27 FEB 2002

FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
31671-176438

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
Unassigned 10/069541

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/05545	August 18, 2000	August 27, 1999

TITLE OF INVENTION
HIGH-AFFINITY CHOLINE TRANSPORTER

APPLICANT(S) FOR DO/EO/US
Tatsuya HAGA and Takashi OKUDA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S C. 371(c)(2)).
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau. (attach form IB 308)
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording A separate cover sheet in compliance with 37 CFR 3.28 and 3 31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U S C 1 821 - 1 825
18. ☐ A second copy of the published international application under 35 U S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U S C 154(d)(4).
20. ☒ Other items or information: Paper copy of Sequence Listing (29 pages) and Petition for Full-tone Photographs ($130 00)
20a. ☐ **For purposes of examination, please insert the annexes to the IPER, so that the application will comprise the following pages of the English translation:**
Specification: Original pages Amended pages
Claims: Original claims Amended claims

10/069141 .022702

JC13 Rec'd PCT/PTO 27 FEB 2002

U.S. APPLICATION NO. (if known see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Unassigned	PCT/IB00/01345	31671-17648

21. ☒ The following fees are submitted

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5):

Neither international preliminary examination fee (37 CFR 1 482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040 00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00

International preliminary examination fee (37 CFR 1 482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $740.00

International preliminary examination fee (37 CFR 1 482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of $130 00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	98 - 20 =	78	x $18.00	$1404 00	
Independent claims	26 - 3 =	23	x $84.00	$1932.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$4226.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$	
SUBTOTAL =				$4226 00	
Petition under 37 CFR 1.84 processing fee of **$130.00**.				$130.00	
TOTAL NATIONAL FEE =				$4356 00	
Fee for recording the enclosed assignment (37 CFR 1 21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3 31) **$40.00** per property			+	$40 00	
TOTAL FEES ENCLOSED =				$4396 00	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 4396 00 to cover the above fees is enclosed

b. ☐ Please charge my Deposit Account No _____ in the amount of $ _____ to cover the above fees

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No 22-0261. A duplicate copy of this sheet is enclosed

d. ☐ Fees are to be charged to a credit card **WARNING:** Information on this form may become public Credit card information should not be included on this form Provide credit card information and authorization on PTO-2038

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

VENABLE
P.O. Box 34385
Washington D C 20043-9998
Phone No. 202-962-4800
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SIGNATURE

Robert Kinberg
NAME

26,924
REGISTRATION NUMBER

PC Docs No **354835**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Tatsuya HAGA et al.

Appl. No. Unassigned

Filed: February 27, 2002

For: HIGH-AFFINITY CHOLINE TRANSPORTER

Int'l. Appln. No.: PCT/JP00/05545

Int'l. Filing Date: August 18, 2000

Atty. Docket No. 31671-176438

Customer No.

26694

PATENT TRADEMARK OFFICE

PETITION UNDER 37 CFR 1.84

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a Petition under Rule 84 (a)(2) to accept color photographs and illustrations in the above identified application for Figures 9 and 10. On information and belief, the claimed invention and explanation thereof is technically difficult to illustrate by black and white line drawings. On information and belief, color photographs and illustrations are necessary because they are the only practicable medium for illustrating the claimed invention and explanation thereof in a manner that will facilitate an understanding of the claimed invention.

In compliance with the Rule for submission of color photographs and illustrations, the formal drawings concurrently submitted include three (3) sets of color photographs. Additionally, the application includes the statement required by Rule 1.84 (a)(2) as the first paragraph in the Brief Description of the Drawing.

A check covering the $130.00 Petition fee required by Rule 17(h) is attached. If no fee is attached or if the check is for an insufficient amount, authorization is given to charge our Deposit Account No. 22-0261 with any balance due and to credit our account with any overpayment.

03/04/2002 NNGUYEN 00000004 10069541

04 FC:122 130.00 OP

Applicant(s): Tatsuya HAGA et al.

In view of the above, it is requested that color photographs be permitted in the present application.

Respectfully submitted,

Date: 2/27/02

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Tatsuya HAGA et al.	Int'l. Appln. No.: PCT/JP00/05545
Appl. No. Unassigned	Int'l. Filing Date: August 18, 2000
Filed: February 27, 2002	Atty. Docket No. 31671-176438
For: HIGH-AFFINITY CHOLINE TRANSPORTER	Customer No. 26694 PATENT TRADEMARK OFFICE

Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to calculation of the fees, please amend claims 24-27, 29-33, 35-38, 40-48, 50-52 and 56-63 attached to the specification as follows:

24. (Amended) A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 16.

25. (Amended) A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 18.

26. (Amended) A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 20.

27. (Amended) A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 22.

29. (Amended) An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 16.

30. (Amended) An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 18.

31. (Amended) An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 20.

32. (Amended) An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 22.

33. (Amended) The antibody according to claim 28, wherein the antibody is a monoclonal antibody.

35. (Amended) A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 16.

36. (Amended) A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 18.

37. (Amended) A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 20.

38. (Amended) A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 22.

40. (Amended) A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 16.

41. (Amended) A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its

chromosome, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 18.

42. (Amended) A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 20.

43. (Amended) A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 22.

44. (Amended) The non-human animal according to claim 39, wherein the non-human animal is a mouse or a rat.

45. (Amended) A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene according to claim 8 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome.

46. (Amended) A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene according to claim 8 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome, wherein the cell having high-affinity choline transporter activity is integrated with the gene in its chromosome, and stably shows high-affinity choline transporter activity.

47. (Amended) A cell having high-affinity choline transporter activity being obtainable by the preparing method of a cell having high-affinity choline transporter activity according to claim 45.

48. (Amended) A screening method of a promoter or a suppressor of high-affinity choline transporter activity characterized in measuring/evaluating high-affinity choline transporter activity of the protein having high-affinity choline transporter activity according to claim 14 in the presence of a subject material.

50. (Amended) A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the host cell containing an expression system which can express a protein having high-affinity choline transporter activity according to claim 34.

51. (Amended) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 48, wherein the protein having high-affinity choline transporter activity is a recombinant protein.

52. (Amended) A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell obtained from the non-human animal according to claim 39 is cultivated in vitro in the presence of a subject material; the activity and/or the expression

amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated.

56. (Amended) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 52, wherein the non-human animal is a mouse or a rat.

57. (Amended) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 48.

58. (Amended) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 48.

59. (Amended) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein according to claim 14.

60. (Amended) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the protein according to claim 14.

61. (Amended) A diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter characterized in comparing a DNA sequence encoding a high-affinity choline transporter in a sample to a DNA sequence encoding the protein according to claim 19.

62. (Amended) A diagnostic probe for Alzheimer's disease comprising a whole or a part of an antisense strand of DNA or RNA encoding the protein according to claim 19.

63. (Amended) A diagnostic drug for Alzheimer's disease characterized in containing the diagnostic probe according to claim 62.

Please add the following new claims:

64. (New) A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the DNA according to claim 9 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome.

65. (New) A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the DNA according to claim 9 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome, wherein the cell having high-affinity choline transporter activity is integrated with the DNA in its chromosome, and stably shows high-affinity choline transporter activity.

66. (New) A cell having high-affinity choline transporter activity being obtainable by the preparing method of a cell having high-affinity choline transporter activity according to claim 46.

67. (New) A cell having high-affinity choline transporter activity being obtainable by the preparing method of a cell having high-affinity choline transporter activity according to claim 65.

68. (New) A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the cell having high-affinity choline transporter activity according to claim 47.

69. (New) A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the cell having high-affinity choline transporter activity according to claim 64.

70. (New) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 49, wherein the protein having high-affinity choline transporter activity is a recombinant protein.

71. (New) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 53, wherein the non-human animal is a mouse or a rat.

72. (New) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 54, wherein the non-human animal is a mouse or a rat.

73. (New) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 55, wherein the non-human animal is a mouse or a rat.

74. (New) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 49.

75. (New) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 52.

76. (New) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 53.

77. (New) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 54.

78. (New) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 55.

79. (New) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 49.

80. (New) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 52.

81. (New) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 53.

82. (New) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 54.

83. (New) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to claim 55.

84. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 57 as an active component.

85. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or

expression of a protein having high-affinity choline transporter activity according to claim 74 as an active component.

86. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 75 as an active component.

87. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 76 as an active component.

88. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 77 as an active component.

89. (New) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 78 as an active component.

90. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline

transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 58 as an active component.

91. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 79 as an active component.

92. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 80 as an active component.

93. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 81 as an active component.

94. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 82 as an active component.

95. (New) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline

transporter, and containing the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 83 as an active component.

96. (New) A diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter characterized in comparing a DNA sequence encoding a high-affinity choline transporter in a sample to a DNA sequence encoding the protein according to claim 20.

97. (New) A diagnostic probe for Alzheimer's disease comprising a whole or a part of an antisense strand of DNA or RNA encoding the protein according to claim 20.

98. (New) A diagnostic drug for Alzheimer's disease characterized in containing the antibody according to claim 28.

REMARKS

This Preliminary Amendment is made to eliminate multiple claim dependency. Examination on the merits of the application is requested. A marked up version showing the changes made to the claims is attached.

Respectfully submitted,

Date: 2/27/02

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MARKED-UP VERSION OF THE CLAIMS

24. (Amended) [The fusion protein according to claim 23] A fusion protein being constructed by expressing a cDNA encoding fusion protein of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim [15 or] 16.

25. (Amended) [The fusion protein according to claim 23] A fusion protein being constructed by expressing a cDNA encoding fusion protein of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim [17 or] 18.

26. (Amended) [The fusion protein according to claim 23] A fusion protein being constructed by expressing a cDNA encoding fusion protein of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim [19 or] 20.

27. (Amended) [The fusion protein acording to claim 23] A fusion protein being constructed by expressing a cDNA encoding fusion protein of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim [21 or] 22.

29. (Amended) [The antibody according to claim 28] An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the

protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim [15 or] 16.

30. (Amended) [The antibody according to claim 28] An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim [17 or] 18.

31. (Amended) [The antibody according to claim 28] An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim [19 or] 20.

32. (Amended) [The antibody according to claim 28] An antibody which specifically binds to a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim [21 or] 22.

33. (Amended) The antibody according to [any one of claims 28 to 32] claim 28, wherein the antibody is a monoclonal antibody.

35. (Amended) [The host cell according to claim 34] A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim [15 or] 16.

36. (Amended) [The host cell according to claim 34] A host cell containing an expression system which can express a protein having high-affinity choline transporter

activity, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim [17 or] 18.

37. (Amended) [The host cell according to claim 34] A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim [19 or] 20.

38. (Amended) [The host cell according to claim 34] A host cell containing an expression system which can express a protein having high-affinity choline transporter activity, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim [21 or] 22.

40. (Amended) [The non-human animal according to claim 39] A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim [15 or] 16.

41. (Amended) [The non-human animal according to claim 39] A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim [17 or] 18.

42. (Amended) [The non-human animal according to claim 39] A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein

having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim [19 or] 20.

43. (Amended) [The non-human animal according to claim 39] A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim [21 or] 22.

44. (Amended) The non-human animal according to [any one of claims 39 to 43] claim 39, wherein the non-human animal is a mouse or a rat.

45. (Amended) A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene according to [any one of claims 8 to 10] claim 8 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome.

46. (Amended) [The preparing method of a cell having high-affinity choline transporter activity according to claim 45] A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene according to claim 8 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome, wherein the cell having high-affinity choline transporter activity is integrated with the gene [or the DNA according to any one of claims 8 to 10] in its chromosome, and stably shows high-affinity choline transporter activity.

47. (Amended) A cell having high-affinity choline transporter activity being obtainable by the preparing method of a cell having high-affinity choline transporter activity according to claim 45 [or 46].

48. (Amended) A screening method of a promoter or a suppressor of high-affinity choline transporter activity characterized in measuring/evaluating high-affinity choline transporter activity of the protein having high-affinity choline transporter activity [according to any one of claims 14 to 22] claim 14 in the presence of a subject material.

50. (Amended) [The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 49] A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the host cell containing an expression system which can express a protein having high-affinity choline transporter activity according to [any one of claims 34 to 38] claim 34[, or is the cell having high-affinity choline transporter activity according to claim 47].

51. (Amended) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to [any one of claims 48 to 50] claim 48, wherein the protein having high-affinity choline transporter activity is a recombinant protein.

52. (Amended) A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell obtained from the non-human animal according to [any one of claims 39 to 44] claim 39 is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated.

56. (Amended) The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to [any one of clams 52 to 55] claim 52, wherein the non-human animal is a mouse or a rat.

57. (Amended) A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to [any one of claims 48 to 56] claim 48.

58. (Amended) A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to [any one of claims 48 to 56] claim 48.

59. (Amended) A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein according to [any one of claims 14 to 22,] claim 14 [and/or the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 57] as an active component.

60. (Amended) A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the protein according to [any one of claims 14 to 22,] claim 14 [and/or the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 58] as an active component.

61. (Amended) A diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter characterized in comparing a DNA sequence encoding a high-affinity choline transporter in a sample to a DNA sequence encoding the protein according to claim 19 [or 20].

62. (Amended) A diagnostic probe for Alzheimer's disease comprising a whole or a part of an antisense strand of DNA or RNA encoding the protein according to claim 19 [or 20].

63. (Amended) A diagnostic drug for Alzheimer's disease characterized in containing the diagnostic probe according to claim 62 [and/or the antibody according to any one of claims 28 to 33].

10/prts

SPECIFICATION

TITLE OF THE INVENTION

HIGH-AFFINITY CHOLINE TRANSPORTER

Technical Field

This invention relates to a protein having high-affinity choline transporter activity, a gene encoding said protein and the use of the same.

Prior Art

The autonomic nervous system which spreads to organs throughout a body and regulates the most basic functions of living organism including energy metabolism, circulation, respiration and reproduction along with endocrine system, is classified into the sympathetic and parasympathetic nervous systems. All autonomic nerve fibers excluding postganglionic fibers of the sympathetic nerve, motor nerve fiber, and sudoriferous gland/blood vessel dilative fiber in the sympathetic nerve are cholinergic, and acetylcholine is vital for the function of the autonomic nerve and the motor nerve. It has been known that the cholinergic neuron, being observed also in the brain, is important for recognizing function of the brain and that it degenerates after the onset of Alzheimer's disease. In the cholinergic neuron, because of lack of biosynthetic ability for choline, choline, an acetylcholine decomposition product, is taken up into a cell by a high-affinity choline transporter at the presynaptic terminals to be reused for synthesizing acetylcholine. The high-affinity choline uptake is a rate-limiting step for acetylcholine

synthesis and is presumed to regulate the efficiency of synaptic transmission (J. Neurochem. 18, 781-798, 1971, Science 178, 626-628, 1972, Biochem. Biophys. Acta 291, 564-575, 1973, Mol. Pharmacol. 9, 630-639, 1973, J. Pharmacol. Exp. Ther. 192, 86-94, 1975, J. Neurochem. 30, 15-21, 1978, J. Neurochem. 44, 11-24, 1985, J. Neurochem. 60, 1191-1201, 1993, J. Neurochem. 20, 581-593, 1973, Eur. J. Pharmacol. 102, 369-370, 1984). To date, most of cDNAs of transporters for major neurotransmitters have been isolated, however, a cDNA of the high-affinity choline transporter, which is physiologically important, has not been identified.

Disclosure of the Invention

So far, the existence of a protein being localized in the cholinergic neuron and having a function of taking up choline, a precursor of acetylcholine, into a cell has been expected, but molecular properties of said protein, a high-affinity choline transporter, have been unknown. An object of the present invention is to provide a physiologically important protein having the high-affinity choline transporter activity, a gene which encodes the protein, and a screening method of a high-affinity choline transporter activity promoter using the protein, the gene and the like.

The inventors have conducted intensive study to attain the above-mentioned object: with information of genomic project (Science 282, 2012-2018, 1998), Na^+-dependent transporter cDNAs being expected from the genomic sequence of a nematode (C. elegans) were cloned one by one, and the high-affinity choline uptake activity of each cDNA was examined in the oocyte expression system of Xenopus, and the cDNA of nematode

high-affinity choline transporter (cho-1) was identified on the basis of the above examination, then homologous molecules (CHT1) were cloned from rat spinal cord by using the homology of a base sequence to the cDNA as an index. This CHT1 had no homology to neurotransmitter transporters (J. Neurochem. 71, 1785-1803, 1998), but had 20 to 25% homology to molecules which belong to Na^+-dependent glucose transporter family (Nature 330, 379-381, 1987).

Northern blot analysis revealed that transcripts of CHT1 were confirmed only in spinal cord, basal forebrain, corpus striatum and brain stem, and CHT1 seemed to be expressed in cholinergic neurons. Accordingly, CHT1 was expressed in oocytes of Xenopus. As a result, choline uptake activity that is Na^+-dependent and completely inhibited by hemicholinium-3 was observed. These results indicate that CHT1 has high-affinity choline transporter activity. Further, the inventors have cloned choline transporter cDNAs derived from a human and from a mouse, and determined their base sequences, and have confirmed that their expression products have high-affinity choline uptake activity. The present invention has thus completed.

The present invention relates to a gene which encodes a protein having high-affinity choline transporter activity (claim 1), a gene which encodes a protein (a) or (b) described below; (a) a protein comprising an amino acid sequence represented by Seq. ID No. 2, (b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.2, and having high-affinity choline transporter activity (claim 2), DNA containing a base sequence represented

by Seq. ID No. 1 or its complementary sequence and a part or a whole of these sequences (claim 3), DNA derived from a nematode which hybridizes with DNA comprising a gene according to claim 3 under a stringent condition, and encodes a protein having high-affinity choline transporter activity (claim 4), a gene which encodes a protein (a) or (b) described below; (a) a protein comprising an amino acid sequence represented by Seq. ID No. 4, (b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.4, and having high-affinity choline transporter activity (claim 5), DNA containing a base sequence represented by Seq. ID No. 3 or its complementary sequence and a part or a whole of these sequences (claim 6), DNA derived from a rat which hybridizes with DNA comprising a gene according to claim 6 under a stringent condition, and encodes a protein having high-affinity choline transporter activity (claim 7), a gene which encodes a protein (a) or (b) described below; (a) a protein comprising an amino acid sequence represented by Seq. ID No. 6, (b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having high-affinity choline transporter activity (claim 8), DNA containing a base sequence represented by Seq. ID No. 5 or its complementary sequence and a part or a whole of these sequences (claim 9), DNA derived from a human which hybridizes with DNA comprising a gene according to claim 9 under a stringent condition, and encodes a protein having high-affinity choline transporter activity (claim 10), a gene which encodes a protein (a) or (b) described below; (a) a protein comprising an amino acid sequence represented by Seq.

ID No. 8, (b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.8, and having high-affinity choline transporter activity (claim 11), DNA containing a base sequence represented by Seq. ID No. 7 or its complementary sequence and a part or a whole of these sequences (claim 12), and DNA derived from a mouse which hybridizes with DNA comprising a gene according to claim 12 under a stringent condition, and encodes a protein having high-affinity choline transporter activity (claim 13).

The present invention also relates to a protein having high-affinity choline transporter activity (claim 14), a protein comprising an amino acid sequence represented by Seq. ID No. 2 (claim 15), a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.2, and having nematode high-affinity choline transporter activity (claim 16), a protein comprising an amino acid sequence represented by Seq. ID No. 4 (claim 17), a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.4, and having rat high-affinity choline transporter activity (claim 18), a protein comprising an amino acid sequence represented by Seq. ID No. 6 (claim 19), a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having human high-affinity choline transporter activity (claim 20), a protein comprising an amino acid sequence represented by Seq. ID No. 8 (claim 21), and a protein comprising an amino acid

sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.8, and having mouse high-affinity choline transporter activity (claim 22).

The present invention further relates to a fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag (claim 23), the fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16 (claim 24), the fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18 (claim 25), the fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20 (claim 26), and the fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22 (claim 27).

The present invention still further relates to an antibody which specifically binds to a protein having high-affinity choline transporter activity (claim 28), the antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16 (claim 29), the antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has rat

high-affinity choline transporter activity according to claim 17 or 18 (claim 30), the antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20 (claim 31), the antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22 (claim 32), and the antibody according to any one of claims 28 to 32, wherein the antibody is a monoclonal antibody (claim 33).

The present invention also relates to a host cell containing an expression system which can express a protein having high-affinity choline transporter activity (claim 34), the host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16 (claim 35), the host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18 (claim 36), the host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20 (claim 37), and the host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22 (claim 38).

The present invention further relates to a non-human animal in which function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or

overexpresses on its chromosome (claim 39), the non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16 (claim 40), the non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18 (claim 41), the non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20 (claim 42), the non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22 (claim 43), and the non-human animal according to any one of claims 39 to 43, wherein the non-human animal is a mouse or a rat (claim 44).

The present invention still further relates to a preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene or the DNA according to any one of claims 8 to 10 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome (claim 45), the preparing method of a cell having high-affinity choline transporter activity according to claim 45, wherein the cell having high-affinity choline transporter activity is integrated with the gene or the DNA according to any one of claims 8 to 10 in its chromosome, and stably shows high-affinity choline transporter activity (claim 46), and a cell having high-affinity choline transporter activity being obtainable by

the preparing method of a cell having high-affinity choline transporter activity according to claim 45 or 46 (claim 47).

The present invention also relates to a screening method of a promoter or a suppressor of high-affinity choline transporter activity characterized in measuring/evaluating high-affinity choline transporter activity of the protein having high-affinity choline transporter activity according to any one of claims 14 to 22 in the presence of a subject material (claim 48), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated (claim 49), the screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 49, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the host cell containing an expression system which can express a protein having high-affinity choline transporter activity according to any one of claims 34 to 38, or is the cell having high-affinity choline transporter activity according to claim 47 (claim 50), the screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to any one of claims 48 to 50, wherein the protein having high-affinity choline

transporter activity is a recombinant protein (claim 51), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell obtained from the non-human animal according to any one of claims 39 to 44 is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated (claim 52), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal and then evaluating the activity and/or the expression amount of a protein having high-affinity choline transporter activity (claim 53), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, and then evaluating the activity and/or the expression amount of a protein having high-affinity choline transporter activity (claim 54), a screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, and then evaluating the activity and/or the expression amount of

a protein having high-affinity choline transporter activity in comparison with the case using wild-type non-human animal (claim 55), and the screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to any one of claims 52 to 55, wherein the non-human animal is a mouse or a rat (claim 56).

The present invention further relates to a material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to any one of claims 48 to 56 (claim 57), a material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to any one of claims 48 to 56 (claim 58), a medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein according to any one of claims 14 to 22, and/or the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 57 as an active component (claim 59), and a medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the protein according to any one of claims 14 to 22, and/or the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 58 as an active component (claim 60).

The present invention still further relates to a diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter characterized in comparing a DNA sequence encoding a high-affinity choline transporter in a sample to a DNA sequence encoding the protein according to claim 19 or 20 (claim 61), a diagnostic probe for Alzheimer's disease comprising a whole or a part of an antisense strand of DNA or RNA encoding the protein according to claim 19 or 20 (claim 62), and a diagnostic drug for Alzheimer's disease characterized in containing the diagnostic probe according to claim 62 and/or the antibody according to any one of claims 28 to 33 (claim 63).

Brief Explanation of the Drawings

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Fig. 1 is a view showing the result of [^{3}H] choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3 cRNA) or water.

Fig.2 is a view showing the result of the effect of Na^+ on choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3 cRNA) or water.

Fig.3 is a view showing the result of the HC3-induced inhibition of choline uptake of oocytes from Xenopus of the present invention being injected with nematode cho-1 (C48D1.3

cRNA) or water.

Fig.4 is a view showing amino acid sequences of rat CHT1 and nematode CHO-1 of the present invention respectively.

Fig.5 is a view showing the distribution of neurons expressing cho-1::gfp of the present invention in the nervous system of nematode.

Fig.6 is a view showing the phylogenetic tree of Na^+-dependent glucose transporter family.

Fig.7 is a view showing an expected topology of rat CHT1 of the present invention.

Fig.8 is a view showing the result of Northern blot analysis of CHT1 mRNA transcript in rat tissue of the present invention.

Fig.9 is a view showing the result of *in situ* hybridization analysis of CHT1 transcript in a rat brain of the present invention.

Fig.10 is a view showing the result of *in situ* hybridization analysis of CHT1 transcript in a spinal cord of the present invention.

Fig.11 is a view showing the result of [^{3}H] choline uptake of oocytes from Xenopus of the present invention being injected CHT1 cRNA of the present invention or water.

Fig.12 is a view showing the effect of choline concentration on choline uptake in CHT1 of the present invention.

Fig.13 is a view showing the result of HC3-induced inhibition of choline uptake of CHT1 of the present invention.

Fig.14 is a view showing the result of Na^+- and Cl^--dependent choline uptake of CHT1 of the present invention.

Fig.15 is a view showing the result of [^{3}H] HC3 binding

to the membrane prepared from COS7 cells being introduced with CHT1 cDNA of the present invention or vector pcDNA 3.1 separately.

Fig.16 is a view showing the result of saturation analysis of specific [^{3}H] HC3 binding to the membrane prepared from COS7 cells being introduced with CHT1 cDNA of the present invention or vector pcDNA 3.1 separately.

Fig.17 is a view showing the result of displacement of specific [^{3}H] HC3 binding by HC3 of the present invention, choline (Cho), acetylcholine (ACh).

Best Mode for Carrying out the Invention

The cDNA of nematode high-affinity choline transporter of the present invention, being described in Seq. ID No. 1, can be obtained by injecting each cRNA prepared from candidate full-length cDNAs, which are expected as a member of Na^+-dependent transporter family according to C. elegans genome project, into oocytes of Xenopus, and examining the uptake of choline. The high-affinity uptake of choline in brain synaptosomes of mammals was completely inhibited by 1 μMhemicholinium-3 (HC3) (Ki=10-100 nM), while the low-affinity uptake of choline, which is distributed in every cells, was inhibited only by HC3 with higher concentration (Ki=50 μM). Therefore, the sensitivity to 1 μM HC3 can be used as criteria of high-affinity choline uptake during the process. For example, it is possible to confirm the identification, the expression, and the localization of an object gene from the candidate cDNA of a nematode (C. elegans) as follows.

It has been found that cDNA corresponding to the gene expected as C48D1.3 promotes significant choline uptake, being

inhibited by 1 μM HC3, in the high-affinity choline uptake process. Fig. 1 shows the result of [^{3}H] choline uptake of oocytes from Xenopus being injected with C48D1.3 cRNA or water. In Fig. 1, the closed and the open columns indicate choline uptake in the absence or the presence of 1 μM HC3 respectively, and each column is shown by mean ± SEM (n=6 to 8 oocytes). Fig.2 shows the effect of Na^+ on the choline uptake, and the closed columns indicate choline uptake measured in the standard solution ([Na^+]=100 mM), the open columns indicate choline uptake in the absence of Na^+ (Na^+ was substituted with Li^+). In addition, Fig. 3 shows the inhibition of choline uptake induced by HC3. Based on the above-mentioned Fig. 2 and 3, it is presumed that the uptake is Na^+-dependent, and that Ki of HC3 is 50 nM. The cDNA clone was designated as cho-1 (high-affinity choline transporter-1).

By comparing a base sequence of cDNA and that of genome, cho-1 gene was found to comprise 9 exons. A protein expected from a base sequence of cDNA of cho-1 includes 576 amino acid residues (see Fig. 4), and this protein, being represented by Seq. ID No. 2, can be constructed by a usual method. When the available data base was searched, the amino acid sequences of cho-1 showed weak, but significant homology to members of Na^+-dependent glucose transporter family. Hydrophobic analysis and comparison to other transporters suggest that there is a twelve-transmembrane region (see Fig. 7).

Then, in order to identify cells expressing cho-1 in the nervous system of a nematode (C. elegans), a gene of a green fluorescent protein (GFP) fused with a region 5.1kb upstream from cho-1 gene was introduced into a nematode, and distribution of neurons expressing cho-1::gfp was examined. A photograph

of L1 larva possessing cho-1::gfp reporter DNA at the outside of chromosome is shown as Fig. 5 (scale bar; 50 μm). In Fig. 5, the arrowhead indicates nerve ring. In the ventral nerve cord, GFP is expressed only in cholinergic motor nerve, however, some of DA, DB nerve cells do not express GFP owing probably to deficiency of reporter DNA at the outside of chromosome. It supports the idea that cho-1 is a high-affinity choline transporter of the cholinergic neuron.

The cDNA of rat high-affinity choline transporter of the present invention, being described in Seq. ID No. 3, can be prepared, for example, by a method comprising the steps of: paying attention to cho-1 homologous molecules of vertebrates and searching data base with amino acid sequences expected from cho-1, and identifying one candidate (GenBank accession number: AQ 316435) in human genomic survey sequence (GSS); amplifying cDNA fragments from rat spinal cord cDNA by PCR with degenerate primers on the basis of homology of base sequences between the human genome DNA and cho-1; screening rat spinal cord cDNA library with this fragment, and a positive cDNA clone was obtained. A protein with 580 amino acid residues showing 51% identity and 70% similarity to cho-1 was expected from the base sequence of the longest reading frame (see Fig. 4). This rat cDNA clone was designated as CHT1. In Fig. 4, each amino acid sequence of rat CHT1 and nematode CHO-1 is shown, and the identical and the similar residues are indicated on a black ground and a gray ground respectively. The expected transmembrane region I-XII is underlined. This protein represented by Seq. ID No. 4 can be constructed by a usual method.

The above-mentioned amino acid sequence of CHT1 is significantly homologous to members of Na^+-dependent glucose

transporter family (20 to 25%). The phylogenetic tree of Na^+-dependent glucose transporter family made by neighbor-joining method using a program CLUSTALW of National Institute of Genetics (Mishima, Japan) is shown in Fig. 6. In Fig. 6, the percentage of the identical amino acids, being contained in each protein, to rat CHT1 is shown on the right side. On the other hand, no homology was observed to a yeast choline transporter (J. Biol. Chem. 265, 15996-16003, 1990), a creatine transporter which had been originally reported as a high-affinity choline transporter (Biochem. Biophys. Res. Commun. 198, 637-645, 1994), and other neurotransmitter transporters.

The expected topology of CHT1 is thought to be the same as that of nematode CHO-1 fundamentally. Fig. 7 shows the expected topology of rat CHT1. In Fig. 7, the closed circles indicate the identical residues, the shadowed circles indicate highly conserved residues, and open circles indicate nonsimilar residues. The offshoots indicate the expected glycosylation sites. P among the circles shows the expected parts of phosphorylation induced by protein kinase C.

Next, the distribution of CHT1 mRNA expression was examined by Northern blot analysis and *in situ* hybridization. The expression of transcripts with the length of about 5 kb was confirmed by Northern blot analysis of various tissues of rats. Fig. 8 shows the result of Northern blot analysis of mRNA transcript of CHT1 in rat tissue, and the length of RNA standard (0.24 to 9.5 kb; GIBCO BRL) is exhibited on the left side. As shown in Fig. 8, an abundance of transcripts were confirmed in basal forebrain, brain stem and spinal cord, and a little of those were confirmed in corpus striatum. These tissues are known to contain cholinergic neurons. On the other hand, no

transcript was observed in other regions of the brain or in tissues of non-nervous systems.

Consistent with these results, *in situ* hybridization confirmed the expression of CHT1 mRNA in cell groups of main cholinergic neurons including corpus striatum, cell population in basal forebrain and ventral horn in spinal cord. Fig. 9 and 10 (scale bar; 1 mm) show micrographs of sections in bright-field, which were hybridized with a cRNA probe of an antisense labeled by digoxigenin. These micrographs relate to in situ hybridization analysis of CHT1 transcripts in rat brain and spinal cord. Fig. 9 indicates that mRNA transcripts of CHT1 were detected in vertical and horizontal limbs of the diagonal band (VDB, HDB), medial septal nucleus (MS), caudate and putamen (Cpu), and olfactory tubercle (Tu). Fig. 10 indicates that the expression was observed in ventral horn (VH) in spinal cord. Further, the adjacent section hybridized with a probe of vesicle acetylcholine transporter showed essentially same distribution. This expression distribution is essentially same as the reported distribution of cholineacetyl group transferase or vesicle acetylcholine transporter. These results show that the expression of CHT1 mRNA is limited to cholinergic neurons.

Next, choline uptake of CHT1 was examined by using oocytes of Xenopus. The choline uptake of the oocytes injected with CHT1 cRNA was 2 times to 4 times more than that of controls injected with water. Fig. 11 shows the result of [^{3}H] choline uptake of oocytes of Xenopus injected with CHT1 cRNA or water. In Fig. 11, the open and the closed columns respectively indicate choline uptake in the standard solutions containing 100 mM NaCl or LiCl, and each column is shown by mean ± SEM (n=6

to 8 oocytes). The effect of choline concentration on choline uptake is shown in Fig. 12. In Fig. 12, choline uptake of oocytes injected with water was subtracted from that of oocytes injected with cRNA in order to figure out CHT1-induced choline uptake, and the choline uptake was fitted to Michaelis-Menten curve. As shown in Fig. 12, choline uptake of CHT1 saturated when increasing choline concentration (Km=2.2 ± 0.2 μM, n=3). The Km of endogenous choline uptake of control is higher than 10 μM.

Then, the result of HC3-induced inhibition of choline uptake is shown in Fig. 13. Fig. 13 indicates that choline uptake of CHT1 is completely inhibited by 0.1 μM HC3 (Ki=2-3 nM), whereas 10 μM HC3 induced only slight inhibition in control. As shown in Fig. 14, ion-dependency of choline uptake of CHT1 was examined and found to be Cl^--dependent as well as Na^+-dependent. The closed and the open columns indicate choline uptake of oocytes injected with water and with cRNA respectively (100 mM NaCl in the standard solution is substituted with 100 mM of each salt) shown in the figure. These results indicate that CHT1 has the characteristics expected from high-affinity choline uptake in brain synaptosomes (high-affinity to choline, high sensitivity to HC3, and Na^+-Cl^--dependency) (J. Neurochem. 27, 93-99, 1976).

In addition, [^{3}H] HC3 binding activity of membranes prepared from COS7 cells introduced with CHT1 cDNA and a vector (control) respectively was examined. The result is shown in Fig. 15. As Fig. 15 indicates, Na^+-dependent [^{3}H] HC3 binding was observed in a membrane of a cell where CHT1 was expressed, but not in a control membrane. Subsequently, a saturation analysis was conducted for specific [^{3}H] HC3 binding. As shown

in Fig. 16, equilibrium dissociation constant (Kd) was estimated to be 1.6±0.2μM (n=3). This value was similar to that reported in brain synaptosomes (J. Neurochem. 60, 1191-1201, 1993, Life Sci. 35, 2335-2343, 1984, Brain Res. 348, 321-330, 1985). Further, displacement of specific [^{3}H] HC3 binding by HC3, choline (Cho) and acetylcholine (Ach) was examined. Acetylcholine was measured in the presence of 1 μM physostigmine. The result is shown in Fig. 17. Fig. 17 indicates that specific [^{3}H] HC3 binding was displaced when the concentration of choline was at least about 10 times lower than that of acetylcholine. These results show that CHT1 is a HC3 binding site as well as a high-affinity choline transporter.

The cDNA of human high-affinity choline transporter of the present invention, being represented by Seq. ID No.5, can be prepared, for example, as follows: data base search was conducted with the amino acid sequence of nematode (C. elegans) CHO-1 to find a sequence of specific human genome DNA fragment having significant homology (R-107P12, a clone of human genomic survey sequence; GenBank accession number: AQ316435); a gene-specific primers for PCR were designed based on a base sequence of said DNA fragment; 5'-RACE (rapid amplification of cDNA ends) and 3'-RACE were conducted using Marathon-Ready™ cDNA (Clontech) of human whole brain, together with an attached adapter primer; the obtained PCR product was cloned into a cloning vector for PCR, and a base sequence of inserted DNA was determined. In addition, an amino acid sequence expected from this DNA sequence is represented by Seq. ID No. 6. A protein having human high-affinity choline transporter activity represented by said Seq. ID No. 6 can be constructed by a usual method on the basis of DNA sequence information shown in Seq.

ID No. 5.

The cDNA of mouse high-affinity choline transporter of the present invention, being represented by Seq. ID No.7, can be prepared, for example, as follows: data base search was conducted with the amino acid sequence of nematode (C. elegans) CHO-1 to find a sequence of specific human genome DNA fragment having significant homology (R-107P12, a clone of human genomic survey sequence; GenBank accession number: AQ316435); a gene-specific primer for PCR was designed based on a base sequence of said DNA fragment; 5'-RACE (rapid amplification of cDNA ends) and 3'-RACE were conducted using Marathon-Ready™ cDNA (Clontech) of mouse whole brain, together with an attached adapter primer; the obtained PCR product was cloned into a cloning vector for PCR, and a base sequence of inserted DNA was determined. In addition, an amino acid sequence expected from this DNA sequence is represented by Seq. ID No. 8. A protein having mouse high-affinity choline transporter activity represented by said Seq. ID No. 8 can be constructed by a usual method on the basis of DNA sequence information shown in Seq. ID No. 7.

Examples of a protein having high-affinity choline transporter activity of the present invention include a protein derived from natural materials and a recombinant protein. In addition to the ones represented by Seq. ID Nos. 2, 4, 6 and 8, which are specifically disclosed above, a protein comprising an amino acid sequence wherein one or a few amino acids are deficient, substituted or added in amino acid sequences represented by Seq. ID Nos. 2, 4, 6 and 8, and having high-affinity choline transporter activity is also included. These proteins can be prepared by known methods. Further, examples

of a gene or DNA encoding a protein having high-affinity choline transporter activity of the present invention include, in addition to the ones represented by Seq. ID Nos. 1, 3, 5 and 7, which are specifically disclosed above, a gene or DNA which encodes a protein comprising an amino acid sequence wherein one or a few amino acids are deficient, substituted or added in amino acid sequences represented by Seq. ID Nos. 2, 4, 6 and 8, and having high-affinity choline transporter activity, and DNA which encodes a protein hybridizing with said gene or DNA under a stringent condition and having high-affinity choline transporter activity. These genes and DNAs can be prepared by known methods.

Cholinergic neurons play an extremely important role in learning and memory. The damage of these neurons correlates to severity of dementia. The rate-limiting step in acetylcholine synthesis is presumed to be the uptake of choline, and its activity is controlled by neural activity or various kinds of stimuli. In the brains of patients who suffer Alzheimer's disease, the hyperfunction of high-affinity choline uptake and of HC3 binding activity are observed (Trends Neurosci. 15, 117-122, 1992, Ann. NY Acad. Sci. 777, 197-204, 1996, J. Neurochem. 69, 2441-2451, 1997). Cloning of said gene or DNA encoding a protein having high-affinity choline transporter activity and said protein having high-affinity choline transporter activity is important for elucidating the molecular mechanism of the high-affinity choline transporter and for developing new therapies for Alzheimer's disease.

The fusion protein of the present invention means a substance constructed by binding a protein from a nematode, a rat, a human, a mouse, etc., which has high-affinity choline

transporter activity, to a marker protein and/or a peptide tag. As the marker protein, any conventionally known marker protein can be used and the specific examples are alkaline phosphatase, Fc region of an antibody, HRP, and GFP. Conventionally known peptide tags, such as Myc tag, His tag, FLAG tag, GST tag, are exemplified as specific examples of the peptide tag of the present invention. Said fusion proteins can be constructed by a usual method, and are useful for the purification of a protein having high-affinity choline transporter activity utilizing the affinity between Ni-NTA and His tag, the detection of a protein having high-affinity choline transporter activity, the quantitation of an antibody to a protein having high-affinity choline transporter activity, and as a diagnostic marker for Alzheimer's disease, and an investigational reagent in the field concerned.

As an antibody that specifically combines with a protein having high-affinity choline transporter activity of the present invention, an immunospecific antibody such as a monoclonal antibody, a polyclonal antibody, a chimeric antibody, a single stranded antibody, a humanized antibody and the like are concretely exemplified. Though these antibodies can be constructed by a usual method with the above-mentioned protein having high-affinity choline transporter activity as an antigen, a monoclonal antibody is more preferable among them because of its specificity. Said antibody that specifically binds to a protein having high-affinity choline transporter activity, such as a monoclonal antibody or the like, is useful, for instance, for the diagnosis of Alzheimer's disease, and for elucidation of molecular mechanism of a high-affinity choline transporter.

An antibody to a protein having high-affinity choline transporter activity is developed by administering fragments containing the protein having high-affinity choline transporter activity or its epitope, or cells that express said protein on the surface of the membrane, to animals (preferably excluding human) with usual protocol. For instance, a monoclonal antibody can be prepared by an arbitrary method that brings antibodies developed by cultured materials of continuous cell line, such as hybridoma method (Nature 256, 495-497, 1975), trioma method, human B-cell hybridoma method (Immunology Today 4, 72, 1983), and EBV-hybridoma method (MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

In order to develop a single stranded antibody to the above-mentioned protein having high-affinity choline transporter activity of the present invention, the preparation method of single stranded antibodies (US Patent No. 4,946,778) can be applied. Further, in order to express a humanized antibody, it is possible to use transgenic mice, other mammalian animals or the like, and to isolate and identify the clones that express a protein having high-affinity choline transporter activity with the above-mentioned antibodies, and to purify the polypeptide by affinity chromatography. An antibody to a protein having high-affinity choline transporter activity could be used, in particular, for the diagnosis and the medical treatment of Alzheimer's disease, and the like.

This invention relates to a host cell which contains an expression system that can express said protein having high-affinity choline transporter activity. The gene that encodes a protein having high-affinity choline transporter activity can be introduced into a host cell by a number of methods

described in many standard laboratory manuals such as by Davis et al. (BASIC METHODS IN MOLECULAR BIOLOGY, 1986), and by Sambrook et al. (MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 1989). Examples of those methods include calcium phosphate transfection, DEAE-dextran-mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection. Examples of the host cells include bacterial procaryotic cells such as Escherichia coli, Streptomyces, Bacillus subtilis, Streptococcus, Staphylococcus and the like; fungous cells such as yeast, Aspergillus and the like; insect cells such as drosophila S2, spodptera Sf9 and the like; and animal or plant cells such as L cells, CHO cells, COS cells, HeLa cells, C127 cells, BALB/c3T3 cells (including mutant strains deficient in dihydrofolate reductase, thymidine kinase or the like), BHK21 cells, HEK293 cells, Bowes melanoma cells and the like.

As the expression system, any expression system that can express a protein having high-affinity choline transporter activity in a host cell will suffice. Examples of the expression system include expression systems derived from chromosome, episome and virus, for example, vectors derived from bacterial plasmid, yeast plasmid, papovavirus like SV40, vaccinia virus, adenovirus, chicken pox virus, pseudorabies virus, or retrovirus, vectors derived from bacteriophage, transposon, and the combination of these, for instance, vectors derived from genetic factors of plasmid and of bacteriophage such as cosmid or phagemid. These expression systems may contain a regulatory sequence that acts not only as a promoter

but also as a controller of expressions.

A host cell that contains the above-mentioned expression system, cell membrane of said host cell, and a protein having high-affinity choline transporter activity which is obtainable by the cultivation of said host cell can be used in the screening method of the present invention as hereinafter described. For example, the method of F. Pietri-Rouxel et al. (Eur. J. Biochem., 247, 1174-1179, 1997) or the like can be used as the method to obtain cell membranes, and publicly known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic-interaction chromatography, affinity chromatography, hydroxyapatite chromatography, and lectin chromatography, preferably high-speed liquid chromatography can be used to pick up said protein having high-affinity choline transporter activity from cell cultured material and purify it. As columns used for affinity chromatography, in particular, there are columns to which a protein antibody having anti-high-affinity choline transporter activity is bound, or in case that a normal peptide tag is added to said high-affinity choline transporter, there are columns to which materials having affinity to the peptide tag are bound. Proteins having high-affinity choline transporter activity can be obtained by using these columns.

In the present invention, said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient on its chromosome means a non-human animal wherein a part or a whole of a gene encoding a protein having high-affinity choline transporter activity on chromosome is inactivated by gene mutation such as

disruption, deficiency, substitution, etc. and function of expressing a protein having high-affinity choline transporter activity is lost. In addition, a non-human animal that overexpresses function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome means a non-human animal that produces larger amount of a protein having high-affinity choline transporter activity than a wild-type non-human animal does. Though specific examples of a non-human animal of the present invention include rodents, such as mice, rats and the like, a non-human animal of the present invention is not limited to these animals.

Homozygous non-human animals generated according to Mendelian ratio include a deficient type or an overexpression type for a protein having high-affinity choline transporter activity, and their littermate wild-type, and it is possible to carry out precise comparative experiments in individual level by using the deficient types, the overexpression types and the littermate wild-types of these homozygous non-human animals at the same time. Therefore, it is preferable to use animals of the same species, more preferably the littermates, as the wild-type non-human animals, in other words, the non-human animals being deficient in or overexpressing the function of a gene that encodes a protein having high-affinity choline transporter activity on their chromosome together in, for example, the screening hereinafter described in the present invention. The generating method of the non-human animals being deficient or overexpressing the function of a gene that encodes a protein having high-affinity choline transporter activity on their chromosome will be explained below, with an example of knockout mice and transgenic mice of a protein having

high-affinity choline transporter activity.

For example, a mouse being deficient in the function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome, in other words, a knockout mouse of a protein having high-affinity choline transporter activity on its chromosome can be constructed as follows. A gene that encodes a protein having high-affinity choline transporter activity is screened by using a gene fragment obtained from mouse gene library by a method like PCR. The screened gene that encodes a protein having high-affinity choline transporter activity is subcloned with a viral vector or the like, and specified by DNA sequencing. A target vector is constructed by substituting a whole or a part of a gene of this clone that encodes a protein having high-affinity choline transporter activity with pMC1 neo gene cassette or the like, and by introducing a diphteria toxin A fragment (DT-A) gene, a herpes simplex virus thymidine kinase (HSV-tk) gene or other such genes into 3'-terminal side.

This constructed target vector is linearized and introduced into ES cells by electroporation or the like to induce homologous recombination. The ES cells wherein homologous recombination is induced by an antibiotic such as G418, ganciclovir (GANC) or the like are selected from the homologous recombinants. It is preferable to confirm whether the selected ES cells are the recombinants of the object by Southern blot or the like. A chimeric mouse is constructed by microinjecting a clone of the confirmed ES cells into a blastocyst of a mouse and then transplanting the blastocyst into a recipient mouse. A heterozygous mouse can be obtained by interclossing the chimeric mouse with a wild-type mouse, and

a knockout mouse of a protein having high-affinity choline transporter activity of the present invention can be constructed by interclossing the heterozygous mice. It is possible to confirm whether a knockout mouse of a protein having high-affinity choline transporter activity is constructed, for example, by isolating RNA from the mouse obtained by said method and examining it by Northern blot analysis or the like, or by examining the expression of the mouse by Western blot analysis or the like.

The transgenic mice of a protein having high-affinity choline transporter activity can be generated in following procedures. A transgene is constructed by fusing chicken β-actin, mouse neurofilament, SV40 or other such promoters, and rabbit β-globin, SV40 or other such poly A or introns with cDNA that encodes a protein having high-affinity choline transporter activity. The transgene is microinjected into the pronucleus of a fertilized egg of a mouse, and the obtained egg cell is cultured, then transplanted to the oviduct of a recipient mouse. After rearing up the recipient animal, baby mice that have the above-mentioned cDNA are selected from the mice born from the recipient animal. Thus transgenic mice can be generated. The baby mouse that has cDNA can be selected by extracting crude DNA from a tail or the like of a mouse, then carrying out methods like dot hybridization using the introduced gene that encodes a protein having high-affinity choline transporter activity as a probe, PCR method using a specific primer, and the like.

In addition, cells being useful for gene therapy of Alzheimer's disease and the like can be prepared by using a whole or a part of a gene or DNA that encodes a protein having high-affinity choline transporter activity of the present

invention. As an example of a method for preparing these cells of the present invention, a method wherein a whole or a part of said gene or DNA of the present invention is introduced into a cell being deficient in the function of a gene that encodes a protein having high-affinity choline transporter activity on its chromosome by transfection or the like to obtain a cell having high-affinity choline transporter activity is exemplified. As the cell having high-affinity choline transporter activity, in particular, it is preferable to use a cell wherein said gene or DNA is integrated into a chromosome and high-affinity choline transporter activity is exhibited stably.

By using the above-mentioned gene or DNA that encodes a protein having high-affinity choline transporter activity, a protein having high-affinity choline transporter activity, a fusion protein created by combining a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag, an antibody to a protein having high-affinity choline transporter activity, a host cell which contains an expression system that can express a protein having high-affinity choline transporter activity, a cell having high-affinity choline transporter activity, or the like, it becomes possible to screen a pharmaceutical material useful for the treatment of symptoms as in Alzheimer's disease or the like, in other words, a material that promotes or suppresses the activity or the expression of a high-affinity choline transporter.

Examples of the screening method of the present invention are: a method wherein the high-affinity choline transporter activity of the above-mentioned protein having high-affinity

choline transporter activity of the present invention is measured/evaluated in the presence of a subject material; a method wherein a cell membrane or a cell which expresses a protein having high-affinity choline transporter activity of the present invention is cultivated in vitro in the presence of a subject material, and the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated; and a method wherein a subject material is administered to said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome and/or a wild-type non-human animal and then the activity and/or the expression amount of a protein having high-affinity choline transporter activity of the present invention is measured/evaluated. As said cell membrane or said cell, a cell such as a primary cultured cell obtained from said non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome or a wild-type non-human animal etc., a host cell containing an expression system which can express a protein having high-affinity choline transporter activity of the present invention, a cell having high-affinity choline transporter activity of the present invention, and cell membranes of these cells can be specifically exemplified.

The screening methods with said subject material and said protein having high-affinity choline transporter activity are now specifically explained together with examples, but the screening methods of the present invention are not limited to these examples. Cells expressing a protein having high-affinity choline transporter activity are cultured in the

presence of a subject material, and the increase or the decrease of a protein having high-affinity choline transporter activity expressed on the cell surface after a certain period of cultivation can be immunochemically detected by ELISA or other such method with an antibody that specifically combines to a protein having high-affinity choline transporter activity of the present invention, or can be evaluated by using suppression or promotion of mRNA expression as an index. The mRNA can be detected by methods such as DNA chip, Northern hybridization or the like. Moreover, with a cell to which a gene wherein luciferase or other such reporter genes is linked to downstream of promoter of a gene that encodes high-affinity choline transporter is introduced, the suppression or the promotion of the expression of a gene that encodes a protein having high-affinity choline transporter activity induced by a subject material can be detected by using the activity of said reporter gene as an index.

The present invention further relates a medical constituent being used for medical treatment for a patient who needs promotion of the activity or the expression of a protein having high-affinity choline transporter, or a medical constituent being used for medical treatment for a patient who needs suppression of the activity or the expression of a protein having high-affinity choline transporter, wherein the material contains a protein having high-affinity choline transporter activity, a material which promotes the activity or the expression of a protein having high-affinity choline transporter activity, or a material which suppresses activity or expression of a protein having high-affinity choline transporter activity as an active component. As a protein

having high-affinity choline transporter activity is involved in many biological functions including many pathological ones, it is expected that a compound that can stimulate a protein having high-affinity choline transporter activity and a compound being able to inhibit the function of said protein can be used as pharmaceuticals.

As the material which promotes or suppresses the activity or the expression of a protein having high-affinity choline transporter activity, any material can be used as long as it binds to a protein having high-affinity choline transporter activity, or works on a signal transmitting molecule on upstream, and then promotes the activity or the expression of a protein having high-affinity choline transporter activity or inhibits/antagonizes the activity or the expression of the protein by itself. Specific examples include an antibody, a ligand of a protein having high-affinity choline transporter activity, a fragment of said protein, and an oligonucleotide encoding said fragment, and these materials can be used as pharmaceuticals for treatment, prevention or the like of symptoms observed in the case of Alzheimer's disease or other such diseases, but use of them is not limited to the above examples.

The present invention also relates to a diagnostic method for diseases relating to the activity or the expression of a protein having high-affinity choline transporter activity comprising a comparison of a DNA sequence encoding a protein having high-affinity choline transporter activity in a sample with a DNA sequence encoding a protein having high-affinity choline transporter activity of the present invention. The mutant type of DNA which encodes a protein having high-affinity

choline transporter activity can be detected by finding gene-mutated individuals in DNA level, and this is useful for diagnosis of diseases caused by underexpression, overexpression or mutant expression of a protein having high-affinity choline transporter activity. Specific examples of a sample of said detection include cells of trial subjects, for example, genomic DNA, RNA or cDNA obtained from biopsy of blood, urine, saliva, tissue or the like, however said sample is not limited to these examples. It is also possible to use said sample being amplified by PCR or other such methods. Deficiency and insertion mutation of base sequences can be detected by the size change of the amplified product observed in comparison with normal genotype, and point mutation can be identified by hybridizing amplified DNA with a labeled gene that encodes a protein having high-affinity choline transporter activity. Thus, diagnosis or judgement of symptoms observed in the case of Alzheimer's disease or other such diseases can be made by detecting the mutation of a gene that encodes a protein having high-affinity choline transporter activity.

The present invention further relates to a diagnostic probe for diseases showing symptoms similar to those of Alzheimer's disease or the like comprising a whole or a part of an antisense chain of DNA or RNA encoding a protein having high-affinity choline transporter activity, and a diagnostic drug for diseases showing symptoms similar to those of Alzheimer's disease containing the diagnostic probe and/or an antibody which specifically binds to a protein having high-affinity choline transporter activity of the present invention. Said diagnostic probe is not limited in particular, as long as it comprises a whole or a part of an antisense chain of DNA (cDNA)

or RNA (cRNA) encoding a protein having high-affinity choline transporter activity and being long enough to be a probe (at least 20 bases). In order to make a diagnostic drug for symptoms similar to those of Alzheimer's disease containing said probe and/or an antibody which specifically binds to a protein having high-affinity choline transporter activity of the present invention as active components, it is preferable to dissolve said probe into an appropriate buffer or sterilizing water for preventing said probe from decomposition. Further, it is also possible to diagnose diseases showing symptoms similar to those of Alzheimer's disease by methods using these diagnostic drugs, such as immunostaining (Dev. Biol. 170, 207-222, 1995, J. Neurobiol. 29, 1-17, 1996), in situ hybridization (J. Neurobiol. 29, 1-17, 1996), in situ PCR or the like.

Experimental methods or the like of the above-mentioned various experiments will now be explained in more detail below.

(Cloning of high-affinity choline transporter cDNA)

The candidate cDNA of nematode high-affinity choline transporter was isolated from poly (A)+RNA of nematode mixture from various stages in the development by reverse transcription PCR and 3' RACE. Marathon™ cDNA Amplification Kit (Clontech) was used according to its protocol. A primer for sense direction of PCR was designed at a provisional translation initiating point of a predicted gene based on a DNA base sequence obtained from C. elegans genomic project. The amplified PCR product was subcloned into Nco I (smoothing) site and Not I site of a modified pSPUTK vector (Stratagene), and the base sequence of inserted DNA was determined. CHT1 cDNA of rat was isolated from rat spinal cord cDNA library by using GeneTrapper cDNA

Positive Selection System (GIBCO Bio-Rad Laboratory: GIBCO BRL) according to its protocol. The primer used was designed from the base sequence of a cDNA fragment obtained by degenerated PCR. The obtained cDNA clones were analyzed. Among them, positive clones were selected and subcloned into pSPUTK vector and pcDNA3.1+ vector (Invitrogen Corporation).

(Expression in oocytes of Xenopus)

In the presence of cap analog, cRNA was synthesized in vitro with SP6 or T7 RNA polymerase. 20 to 30 ng capped RNA was microinjected into oocytes (stage V to VI) of Xenopus. The uptake was measured in basically same manner as described previously (Nature 360, 467-471, 1992). Two or three days after the injection of RNA, choline uptake was conducted for 30 to 60 min. with oocytes (6 to 8) in 0.75 ml standard solution (0.01 to 1 μM [^{3}H]-choline, 100 mM NaCl, 2mM KCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 10mM HEPES, 5mM Tris: pH 7.4). The oocytes completing uptake were solubilized with 10% SDS, and the amount of [^{3}H] was measured by a liquid scintillation counter.

(GFP expression construct)

The transcriptional fusion construct of cho-1::gfp was constructed by PCR in same manner as described previously (Gene 212, 127-135, 1998). A gene that encodes a green fluorescent protein (GFP) located on downstream of a nuclear localization signal sequence (NLS) was inserted into a position 3 residues downstream of cho-1 translation initiating point so that the reading frame was fitted. NLS and gfp gene were amplified from pPD104.53 vector. In order to prepare 5.1 kb upstream region of cho-1 translation initiating point, a PCR primer being

designed to encompass the first 3 amino acid residues of cho-1 was used. By the same method as previously described (EMBO J. 10, 3959-3970, 1991), rol-6 (su1006) marker and generated DNA were injected into gonads of a nematode simultaneously.

(Northern blot analysis)

6 μg poly(A)+RNA prepared from various tissues of rats was separated by formaldehyde-agarose electrophoresis, and transferred to a nylon membrane, then hybridized with CHT1 cDNA fragment being labeled with [^{32}P] by random prime method in hybridization solution (solution containing the final concentration of 50% formamido, 5 × SSPE, 5 × Denhardt's solution, 0.5% SDS, 100 μg/ml salmon sperm DNA) at 42° C for 16 hours. The nylon membrane was washed under final condition (0.1 × SSPE, 0.1% SDS: 65° C), and then autoradiography was conducted for 7 days together with an enhancing screen.

(In situ hybridization)

The transcript of an antisense labeled with digoxigenin was synthesized in vitro. Alkaline hydrolysis was repeated for the transcripts until their mean length was prepared to be 200 to 400 b. Cryostat sections of fresh frozen tissue (10 to 20 μm) were used. Hybridization was conducted with labeled cRNA probe (about 1 μg/ml) dissolved in 1 × Denhardt's solution [solution containing the final concentration of 50 mM Tris-HCl (pH 8.0), 2.5 mM EDTA, 0.3 M NaCl, 50% formamido, 10% dextran sulphate, 1 mg/ml E. coli tRNA] at 45° C for 20 hours. Then the sections were washed twice in 2 × SSC/50% formamido and once in 1 × SSC/50% formamido, at 45° C respectively. The hybridized probe was visualized by using anti-digoxigenin Fab fragment

(Boehringer-Mannheim) and NBT/BCIP substrate. The sections were brought into reaction in substrate solution for 24 to 48 hours.

(Binding assay)

[^{3}H] hemicholinium-3 (HC3; 128Ci/mmol) was obtained from NEN Life Science Products. Either pcDNA3.1-CHT1 or pcDNA3.1 was transiently expressed in COS7 cells respectively. TransFast Reagent (Promega) was introduced and used according to the protocol. Membranes were prepared by following steps: homogenizing cells in 0.32 M sucrose; centrifuging the cells for 1 hour at 200,000g; and suspending the precipitate. Binding assay was conducted in basically same manner as described previously. Specific binding amount was calculated by subtracting non-specific binding amount determined in the presence of 10 μM HC3 from the whole binding amount. The Kd value was figured out by analyzing specific [^{3}H] HC3 binding amount from data of saturation binding assay with nonlinear approximation.

Industrial Applicability

The present invention makes it possible to provide a protein having high-affinity choline transporter activity, which is physiologically important, and gene DNA encoding said protein. In addition, by using the said protein and gene DNA, it becomes possible to screen materials being useful for prevention or treatment of Alzheimer's disease, and to prepare cells being useful for gene therapy.

What Is Claimed Is:

1. A gene which encodes a protein having high-affinity choline transporter activity.

2. A gene which encodes a protein (a) or (b) described below;
(a) a protein comprising an amino acid sequence represented by Seq. ID No. 2,
(b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.2, and having high-affinity choline transporter activity.

3. DNA containing a base sequence represented by Seq. ID No. 1 or its complementary sequence and a part or a whole of these sequences.

4. DNA derived from a nematode which hybridizes with DNA comprising a gene according to claim 3 under a stringent condition, and encodes a protein having high-affinity choline transporter activity.

5. A gene which encodes a protein (a) or (b) described below;
(a) a protein comprising an amino acid sequence represented by Seq. ID No. 4,
(b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.4, and having high-affinity choline transporter activity.

6. DNA containing a base sequence represented by Seq. ID No. 3 or its complementary sequence and a part or a whole of these sequences.

7. DNA derived from a rat which hybridizes with DNA comprising a gene according to claim 6 under a stringent condition, and encodes a protein having high-affinity choline transporter activity.

8. A gene which encodes a protein (a) or (b) described below;
(a) a protein comprising an amino acid sequence represented by Seq. ID No. 6,
(b) a protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having high-affinity choline transporter activity.

9. DNA containing a base sequence represented by Seq. ID No. 5 or its complementary sequence and a part or a whole of these sequences.

10. DNA derived from a human which hybridizes with DNA comprising a gene according to claim 9 under a stringent condition, and encodes a protein having high-affinity choline transporter activity.

11. A gene which encodes a protein (a) or (b) described below;
(a) a protein comprising an amino acid sequence represented by Seq. ID No. 8,
(b) a protein comprising an amino acid sequence where one or

a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.8, and having high-affinity choline transporter activity.

12. DNA containing a base sequence represented by Seq. ID No. 7 or its complementary sequence and a part or a whole of these sequences.

13. DNA derived from a mouse which hybridizes with DNA comprising a gene according to claim 12 under a stringent condition, and encodes a protein having high-affinity choline transporter activity.

14. A protein having high-affinity choline transporter activity.

15. A protein comprising an amino acid sequence represented by Seq. ID No. 2.

16. A protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.2, and having nematode high-affinity choline transporter activity.

17. A protein comprising an amino acid sequence represented by Seq. ID No. 4.

18. A protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.4, and having rat

high-affinity choline transporter activity.

19. A protein comprising an amino acid sequence represented by Seq. ID No. 6.

20. A protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.6, and having human high-affinity choline transporter activity.

21. A protein comprising an amino acid sequence represented by Seq. ID No. 8.

22. A protein comprising an amino acid sequence where one or a few amino acids are deficient, substituted or added in the amino acid sequence represented by Seq. ID No.8, and having mouse high-affinity choline transporter activity.

23. A fusion protein being constructed by expressing a cDNA encoding fusion proteins of a protein having high-affinity choline transporter activity and a marker protein and/or a peptide tag.

24. The fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16.

25. The fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has

rat high-affinity choline transporter activity according to claim 17 or 18.

26. The fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20.

27. The fusion protein according to claim 23, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22.

28. An antibody which specifically binds to a protein having high-affinity choline transporter activity.

29. The antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16.

30. The antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18.

31. The antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20.

32. The antibody according to claim 28, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22.

33. The antibody according to any one of claims 28 to 32, wherein the antibody is a monoclonal antibody.

34. A host cell containing an expression system which can express a protein having high-affinity choline transporter activity.

35. The host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16.

36. The host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18.

37. The host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20.

38. The host cell according to claim 34, wherein the protein having high-affinity choline transporter activity has mouse

high-affinity choline transporter activity according to claim 21 or 22.

39. A non-human animal whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome.

40. The non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has nematode high-affinity choline transporter activity according to claim 15 or 16.

41. The non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has rat high-affinity choline transporter activity according to claim 17 or 18.

42. The non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has human high-affinity choline transporter activity according to claim 19 or 20.

43. The non-human animal according to claim 39, wherein the protein having high-affinity choline transporter activity has mouse high-affinity choline transporter activity according to claim 21 or 22.

44. The non-human animal according to any one of claims 39 to 43, wherein the non-human animal is a mouse or a rat.

45. A preparing method of a cell having high-affinity choline transporter activity characterized in introducing the gene or the DNA according to any one of claims 8 to 10 into a cell whose function of a gene which encodes a protein having high-affinity choline transporter activity is deficient on its chromosome.

46. The preparing method of a cell having high-affinity choline transporter activity according to claim 45, wherein the cell having high-affinity choline transporter activity is integrated with the gene or the DNA according to any one of claims 8 to 10 in its chromosome, and stably shows high-affinity choline transporter activity.

47. A cell having high-affinity choline transporter activity being obtainable by the preparing method of a cell having high-affinity choline transporter activity according to claim 45 or 46.

48. A screening method of a promoter or a suppressor of high-affinity choline transporter activity characterized in measuring/evaluating high-affinity choline transporter activity of the protein having high-affinity choline transporter activity according to any one of claims 14 to 22 in the presence of a subject material.

49. A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell membrane or a cell which expresses a protein having high-affinity choline transporter

activity is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell membrane or the cell is measured/evaluated.

50. The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to claim 49, wherein the cell membrane or the cell which expresses a protein having high-affinity choline transporter activity is the host cell containing an expression system which can express a protein having high-affinity choline transporter activity according to any one of claims 34 to 38, or is the cell having high-affinity choline transporter activity according to claim 47.

51. The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression according to any one of claims 48 to 50, wherein the protein having high-affinity choline transporter activity is a recombinant protein.

52. A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in comprising the steps of: a cell obtained from the non-human animal according to any one of claims 39 to 44 is cultivated in vitro in the presence of a subject material; the activity and/or the expression amount of a protein having high-affinity choline transporter activity in the cell is measured/evaluated.

53. A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal and then evaluating the activity and/or the expression amount of a protein having high-affinity choline transporter activity.

54. A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, and then evaluating the activity and/or the expression amount of a protein having high-affinity choline transporter activity.

55. A screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-affinity choline transporter expression characterized in administering a subject material to a non-human animal whose function of a gene encoding a protein having high-affinity choline transporter activity is deficient or overexpresses on its chromosome, and then evaluating the activity and/or the expression amount of a protein having high-affinity choline transporter activity in comparison with the case using wild-type non-human animal.

56. The screening method of a promoter or a suppressor of high-affinity choline transporter activity, or of high-

affinity choline transporter expression according to any one of claims 52 to 55, wherein the non-human animal is a mouse or a rat.

57. A material which promotes activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to any one of claims 48 to 56.

58. A material which suppresses activity or expression of a protein having high-affinity choline transporter activity being obtainable by the screening method according to any one of claims 48 to 56.

59. A medical constituent characterized in being used for a medical treatment for a patient who needs elevation of the activity or enhancement of the expression of a high-affinity choline transporter, and containing the protein according to any one of claims 14 to 22, and/or the material which promotes activity or expression of a protein having high-affinity choline transporter activity according to claim 57 as an active component.

60. A medical constituent characterized in being used for medical treatment for a patient who needs suppression of the activity or the expression of a high-affinity choline transporter, and containing the protein according to any one of claims 14 to 22, and/or the material which suppresses the activity or the expression of a protein having high-affinity choline transporter activity according to claim 58 as an active

component.

61. A diagnostic method for diseases relating to the expression or the activity of a high-affinity choline transporter characterized in comparing a DNA sequence encoding a high-affinity choline transporter in a sample to a DNA sequence encoding the protein according to claim 19 or 20.

62. A diagnostic probe for Alzheimer's disease comprising a whole or a part of an antisense strand of DNA or RNA encoding the protein according to claim 19 or 20.

63. A diagnostic drug for Alzheimer's disease characterized in containing the diagnostic probe according to claim 62 and/or the antibody according to any one of claims 28 to 33.

Abstract

The present invention provides a protein having high-affinity choline transporter activity which is important physiologically, a gene encoding the protein, and a method of screening a material promoting the high-affinity choline transporter activity with the use of the same, and the like. By examining high-affinity choline uptake activity of Na^+-dependent transporter cDNA deduced from the genomic sequence of a nematode (C.elegans) in a Xenopus oocyte expression system, the cDNA (cho-1) of nematode high-affinity choline transporter is identified. Then the cDNA (CHT1) of rat high-affinity choline transporter is cloned from rat spinal cord by using the homology of a base sequence to this cDNA as an index. Similarly, the cDNA of human high-affinity choline transporter is cloned from human genome.

Fig. 1

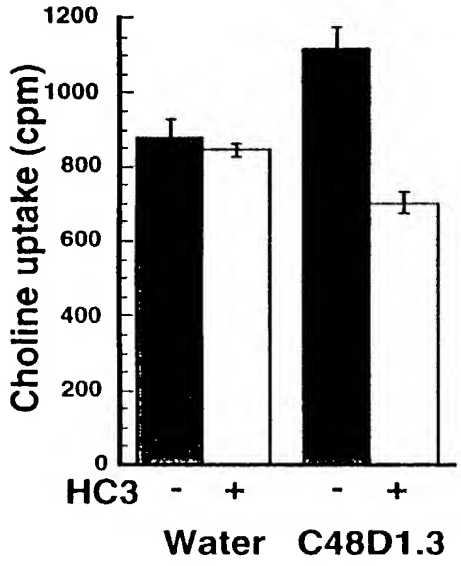


Fig. 2

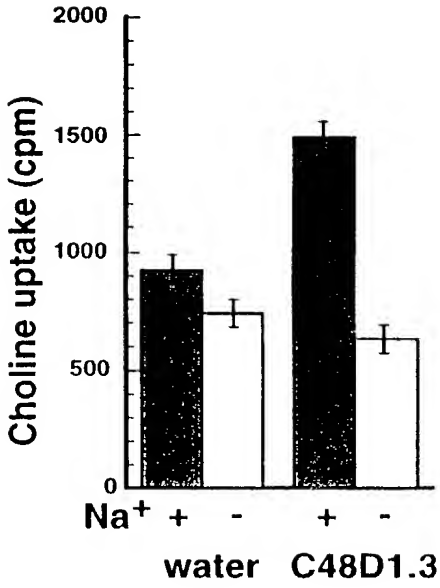


Fig. 3

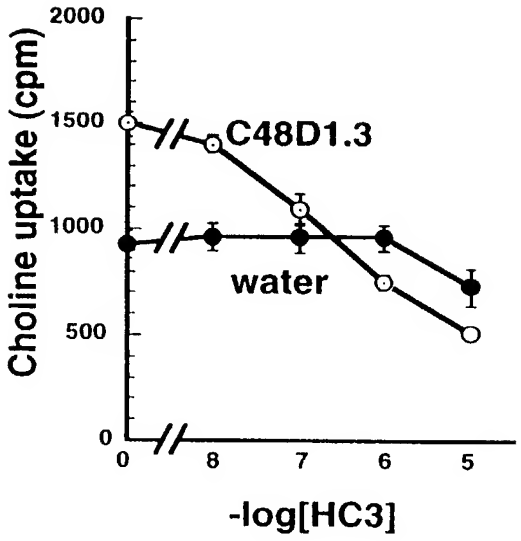
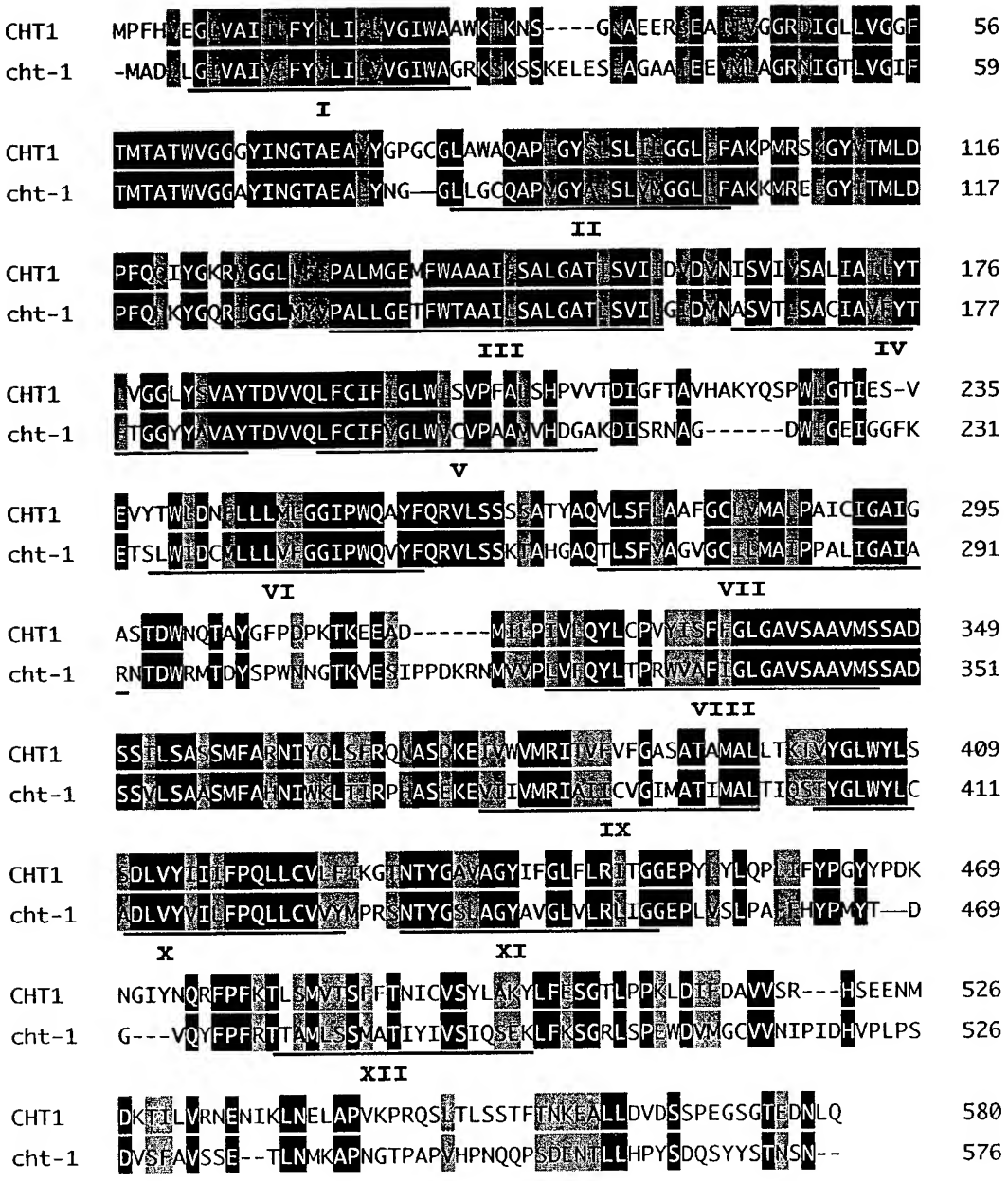


Fig. 4



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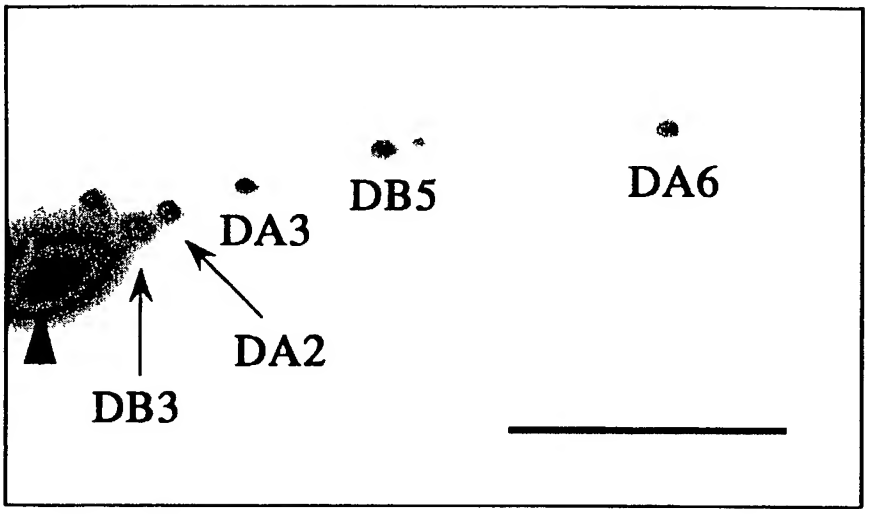
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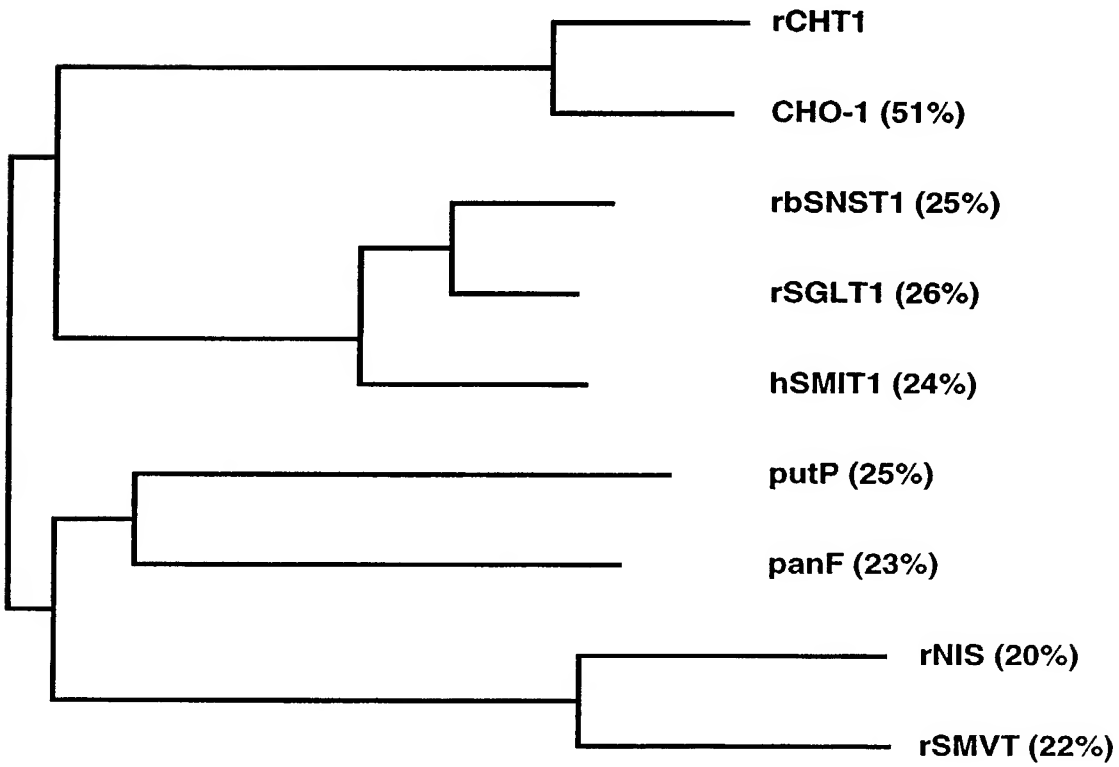
DB5
DA6
DA2
DB3

Fig. 6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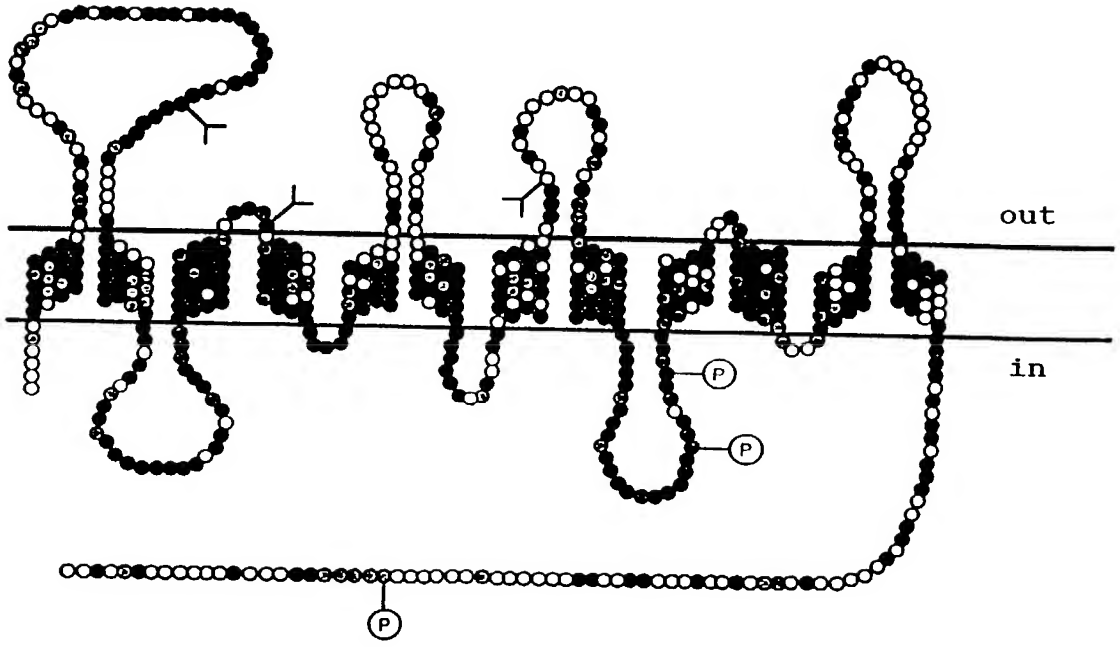
rSGLT1 (26%)
hSMIT1 (24%)
putP (25%)
panF (23%)
rNIS (20%)
rSMVT (22%)

Fig. 7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in
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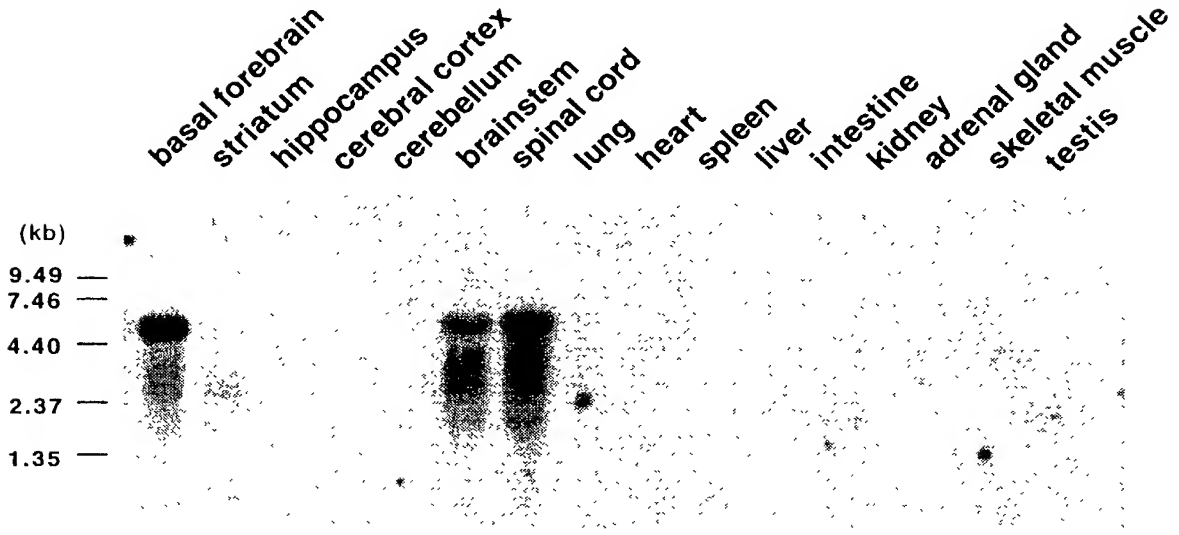
P
P

Fig. 8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striatum
hippocampus
cerebral cortex
cerebellum
brainstem
spinal cord
lung
heart
spleen
liver
intestine
kidney
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9.49
7.46
4.40
2.37
1.35

Fig. 9

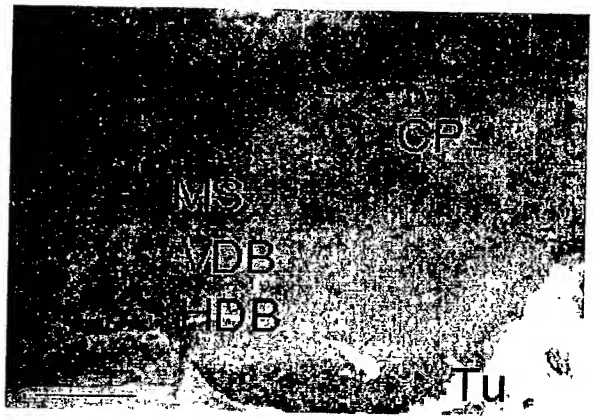


Fig. 10

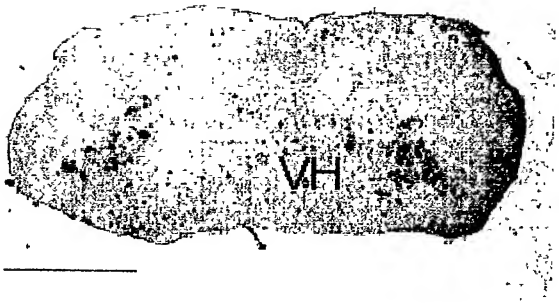
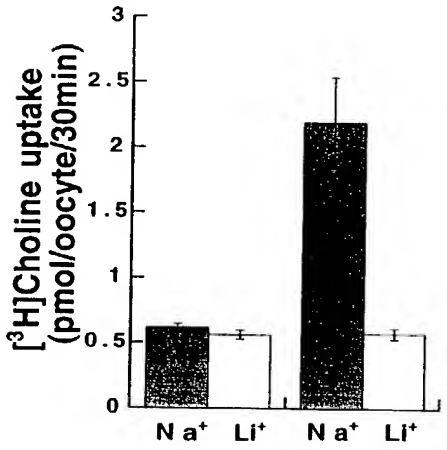


Fig. 11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1.5
1
0.5
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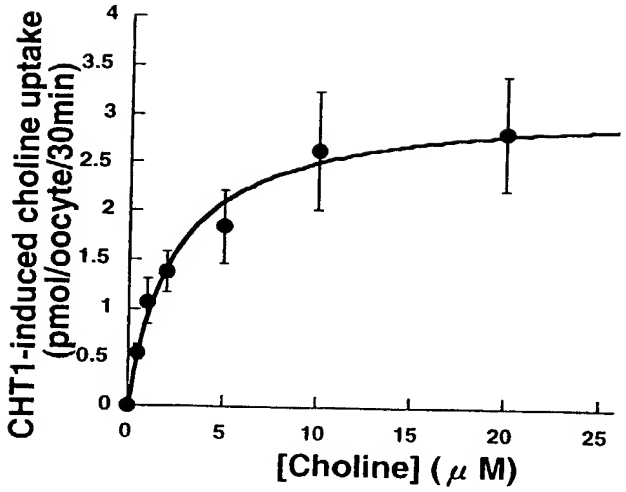
Li+
Na+
Li+

Fig. 12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(pmol/oocyte/30min)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
[Choline] (μ M)

Fig. 13

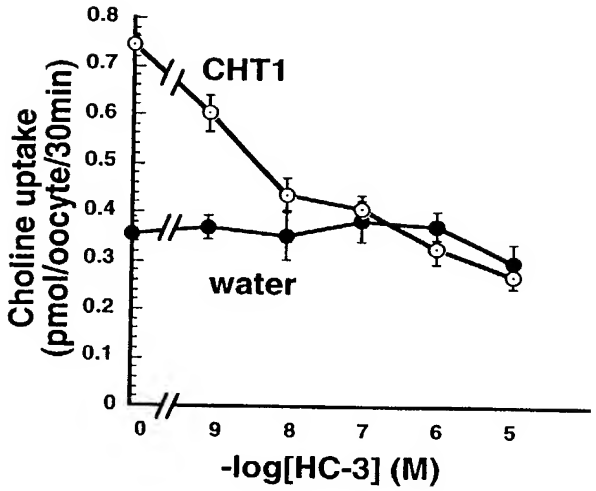
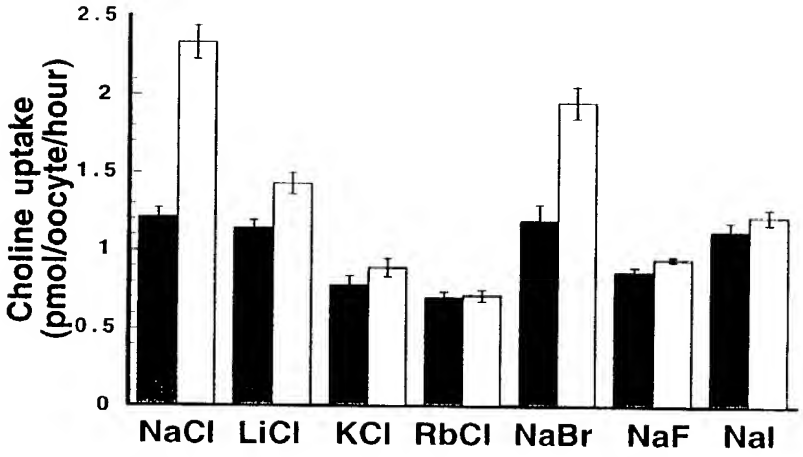


Fig. 14



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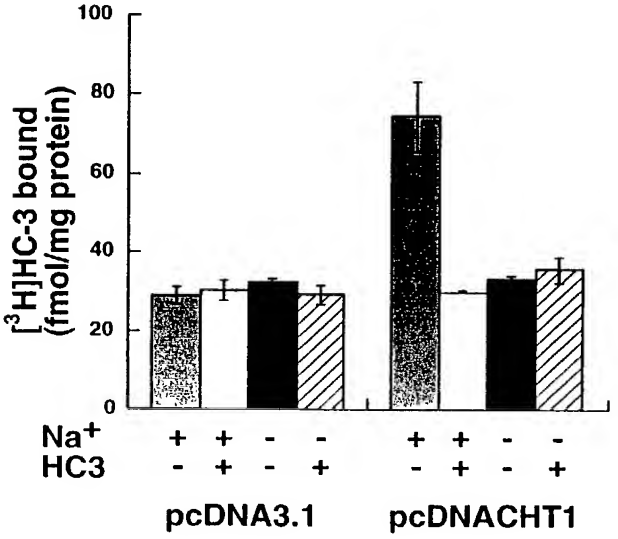
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40
20
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HC3 - + - + - + - +
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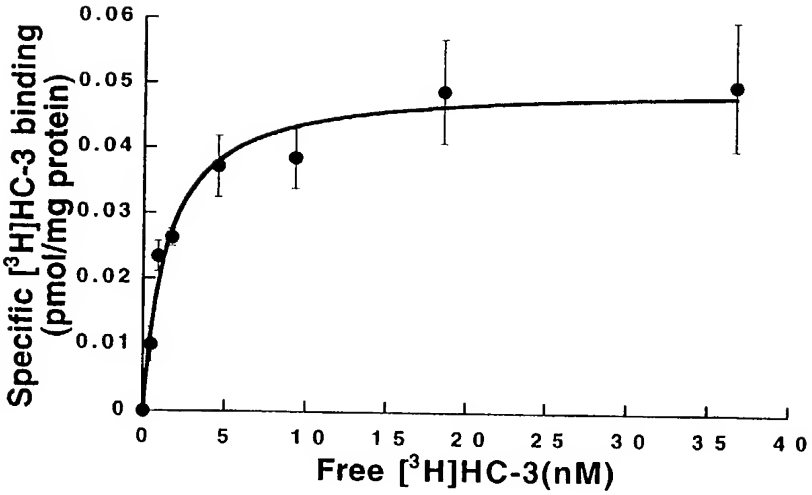
pcDNACHT1

Fig. 16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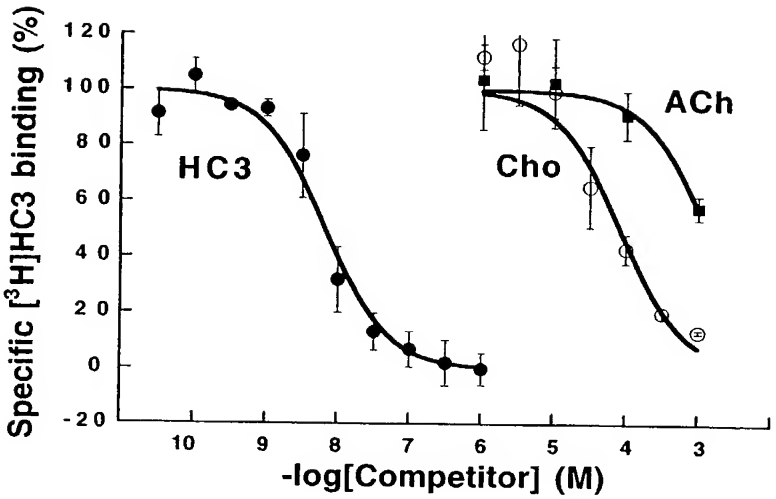
Free [3H]HC-3(nM)

Fig. 17



DECLARATION FOR UNITED STATES PATENT APPLICATION
POWER OF ATTORNEY, DESIGNATION OF CORRESPONDENCE ADDRESS

Attorney Docket
31671-176438 RK

Page 1 of 2

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled HIGH-AFFINITY CHOLINE TRANSPORTER, the specification of which

[ ] is attached hereto.
[ ] was filed on _____, as Application Serial No. _____, Confirmation No. _____, and was amended on _____ [if applicable].
[X] was filed under the Patent Cooperation Treaty on August 18, 2000 Serial No. PCT/JP00/05545, the United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, 119 of any foreign application(s) for patent, utility model, design or inventor's certificate listed below and have also identified below any foreign application(s) for patent, utility model, design or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
Number	Country	Date Filed	Yes	No
11-240642	JAPAN	August 27, 1999	X	
11-368991	JAPAN	December 27, 1999	X	

I hereby appoint the attorneys and agents of VENABLE associated with the following customer number to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

26694
26694
PATENT TRADEMARK OFFICE

VENABLE is located at Suite 1000, 1201 New York Avenue, N.W., Washington, D.C. 20005-3917, Telephone: (202) 962-4800, Telefax: (202) 962-8300. Address all correspondence to VENABLE, Post Office Box 34385, Washington, D.C. 20043-9998.

The undersigned hereby authorizes the U.S. attorneys identified herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorneys identified herein will be so notified by the undersigned.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature: Tatsuya Haga Date: Jan. 9, 2002.
First/Joint Inventor: Tatsuya HAGA
Citizenship: Japanese
Residence and Post Office Address: Room 411, 3-1, Numama 2-chome, Zushi-shi, Kanagawa 249-0004 JAPAN

Attorney Docket: **31671-176438 RK**

DECLARATION FOR UNITED STATES PATENT APPLICATION
POWER OF ATTORNEY, DESIGNATION OF CORRESPONDENCE ADDRESS

Page 2 of 2

Signature: _____ Date: Jan. 9, 2002.
Second/Joint Inventor: Takashi OKUDA
Citizenship: Japanese
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1-6, Hibarigaokakita 4-chome,
Hoya-shi, Tokyo 202-0002 JAPAN JPX

Venable
Washington, D.C. 20005
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115 120 125

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145 150 155 160

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Thr Phe Thr Gly Gly Tyr Tyr Ala Val Ala Tyr Thr Asp Val Val Gln
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Leu Phe Cys Ile Phe Val Gly Leu Trp Val Cys Val Pro Ala Ala Met
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Val His Asp Gly Ala Lys Asp Ile Ser Arg Asn Ala Gly Asp Trp Ile
    210                 215                 220

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Gly Glu Ile Gly Gly Phe Lys Glu Thr Ser Leu Trp Ile Asp Cys Met
225                 230                 235                 240

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                245                 250                 255

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Val Leu Ser Ser Lys Thr Ala His Gly Ala Gln Thr Leu Ser Phe Val
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Ala Gly Val Gly Cys Ile Leu Met Ala Ile Pro Pro Ala Leu Ile Gly
        275                 280                 285

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Val Val Pro Leu Val Phe Gln Tyr Leu Thr Pro Arg Trp Val Ala Phe
                325                 330                 335

att gga ctc ggc gca gtg tcg gct gct gta atg tca tct gca gat tca   1056
Ile Gly Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser
            340                 345                 350

tct gta cta tca gca gca tca atg ttt gct cac aac atc tgg aag ctc   1104
Ser Val Leu Ser Ala Ala Ser Met Phe Ala His Asn Ile Trp Lys Leu
        355                 360                 365

aca att cgc cct cac gcg tct gaa aaa gaa gtg ata att gtg atg aga   1152
Thr Ile Arg Pro His Ala Ser Glu Lys Glu Val Ile Ile Val Met Arg
    370                 375                 380

ata gcc atc atc tgt gtt ggt atc atg gca acc atc atg gca ctt acc   1200
Ile Ala Ile Ile Cys Val Gly Ile Met Ala Thr Ile Met Ala Leu Thr
385                 390                 395                 400

att caa tcc atc tat ggg ctt tgg tat ctt tgt gca gat ttg gtc tac   1248
Ile Gln Ser Ile Tyr Gly Leu Trp Tyr Leu Cys Ala Asp Leu Val Tyr
                405                 410                 415

gtc ata ctc ttc cct caa cta tta tgt gtt gta tat atg cca cgt agc   1296
Val Ile Leu Phe Pro Gln Leu Leu Cys Val Val Tyr Met Pro Arg Ser
            420                 425                 430

aat acg tat ggc tca ttg gct ggc tat gca gtc ggt ctt gtg ctc cgt   1344
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Asn Thr Tyr Gly Ser Leu Ala Gly Tyr Ala Val Gly Leu Val Leu Arg
        435                 440                 445

ttg att gga ggc gag cca ctt gta tcg ctg cca gcg ttc ttc cat tat   1392
Leu Ile Gly Gly Glu Pro Leu Val Ser Leu Pro Ala Phe Phe His Tyr
    450                 455                 460

cca atg tat acg gat ggg gta cag tat ttc cca ttc agg aca act gct   1440
Pro Met Tyr Thr Asp Gly Val Gln Tyr Phe Pro Phe Arg Thr Thr Ala
465                 470                 475                 480

atg tta tct tca atg gct act atc tac att gta tca ata caa tcg gag   1488
Met Leu Ser Ser Met Ala Thr Ile Tyr Ile Val Ser Ile Gln Ser Glu
                485                 490                 495

aag ctg ttc aaa tcg gga cgt ttg tct ccg gag tgg gac gta atg ggt   1536
Lys Leu Phe Lys Ser Gly Arg Leu Ser Pro Glu Trp Asp Val Met Gly
            500                 505                 510

tgt gta gtg aat att ccg ata gat cat gta ccc ctt ccg tca gat gta   1584
Cys Val Val Asn Ile Pro Ile Asp His Val Pro Leu Pro Ser Asp Val
        515                 520                 525

tcg ttt gct gtt agt agt gag acc ttg aat atg aag gct cca aac gga   1632
Ser Phe Ala Val Ser Ser Glu Thr Leu Asn Met Lys Ala Pro Asn Gly
    530                 535                 540

aca ccg gct cca gta cat ccg aac caa cag ccg tct gat gaa aat aca   1680
Thr Pro Ala Pro Val His Pro Asn Gln Gln Pro Ser Asp Glu Asn Thr
545                 550                 555                 560

tta tta cat cca tat tcg gac caa agt tat tat tcc aca aat agc aat   1728
Leu Leu His Pro Tyr Ser Asp Gln Ser Tyr Tyr Ser Thr Asn Ser Asn
                565                 570                 575
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taa 1731

<210> 2
<211> 576
<212> PRT
<213> Caenorhabditis elegans

<400> 2

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Met Ala Asp Leu Leu Gly Ile Val Ala Ile Val Phe Phe Tyr Val Leu
  1               5                  10                  15
Ile Leu Val Val Gly Ile Trp Ala Gly Arg Lys Ser Lys Ser Ser Lys
             20                  25                  30
Glu Leu Glu Ser Glu Ala Gly Ala Ala Thr Glu Glu Val Met Leu Ala
         35                  40                  45
Gly Arg Asn Ile Gly Thr Leu Val Gly Ile Phe Thr Met Thr Ala Thr
     50                  55                  60
Trp Val Gly Gly Ala Tyr Ile Asn Gly Thr Ala Glu Ala Leu Tyr Asn
 65                  70                  75                  80
Gly Gly Leu Leu Gly Cys Gln Ala Pro Val Gly Tyr Ala Ile Ser Leu
                 85                  90                  95
Val Met Gly Gly Leu Leu Phe Ala Lys Lys Met Arg Glu Glu Gly Tyr
            100                 105                 110
Ile Thr Met Leu Asp Pro Phe Gln His Lys Tyr Gly Gln Arg Ile Gly
        115                 120                 125
Gly Leu Met Tyr Val Pro Ala Leu Leu Gly Glu Thr Phe Trp Thr Ala
    130                 135                 140
Ala Ile Leu Ser Ala Leu Gly Ala Thr Leu Ser Val Ile Leu Gly Ile
145                 150                 155                 160
Asp Met Asn Ala Ser Val Thr Leu Ser Ala Cys Ile Ala Val Phe Tyr
                165                 170                 175
Thr Phe Thr Gly Gly Tyr Tyr Ala Val Ala Tyr Thr Asp Val Val Gln
            180                 185                 190
Leu Phe Cys Ile Phe Val Gly Leu Trp Val Cys Val Pro Ala Ala Met
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        195                 200                 205
Val His Asp Gly Ala Lys Asp Ile Ser Arg Asn Ala Gly Asp Trp Ile
    210                 215                 220
Gly Glu Ile Gly Gly Phe Lys Glu Thr Ser Leu Trp Ile Asp Cys Met
225                 230                 235                 240
Leu Leu Leu Val Phe Gly Gly Ile Pro Trp Gln Val Tyr Phe Gln Arg
                245                 250                 255
Val Leu Ser Ser Lys Thr Ala His Gly Ala Gln Thr Leu Ser Phe Val
            260                 265                 270
Ala Gly Val Gly Cys Ile Leu Met Ala Ile Pro Pro Ala Leu Ile Gly
        275                 280                 285
Ala Ile Ala Arg Asn Thr Asp Trp Arg Met Thr Asp Tyr Ser Pro Trp
    290                 295                 300
Asn Asn Gly Thr Lys Val Glu Ser Ile Pro Pro Asp Lys Arg Asn Met
305                 310                 315                 320
Val Val Pro Leu Val Phe Gln Tyr Leu Thr Pro Arg Trp Val Ala Phe
                325                 330                 335
Ile Gly Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser
            340                 345                 350
Ser Val Leu Ser Ala Ala Ser Met Phe Ala His Asn Ile Trp Lys Leu
        355                 360                 365
Thr Ile Arg Pro His Ala Ser Glu Lys Glu Val Ile Ile Val Met Arg
    370                 375                 380
Ile Ala Ile Ile Cys Val Gly Ile Met Ala Thr Ile Met Ala Leu Thr
385                 390                 395                 400
Ile Gln Ser Ile Tyr Gly Leu Trp Tyr Leu Cys Ala Asp Leu Val Tyr
                405                 410                 415
Val Ile Leu Phe Pro Gln Leu Leu Cys Val Val Tyr Met Pro Arg Ser
            420                 425                 430
Asn Thr Tyr Gly Ser Leu Ala Gly Tyr Ala Val Gly Leu Val Leu Arg
        435                 440                 445
Leu Ile Gly Gly Glu Pro Leu Val Ser Leu Pro Ala Phe Phe His Tyr
    450                 455                 460
Pro Met Tyr Thr Asp Gly Val Gln Tyr Phe Pro Phe Arg Thr Thr Ala
465                 470                 475                 480
```

Met Leu Ser Ser Met Ala Thr Ile Tyr Ile Val Ser Ile Gln Ser Glu
485 490 495
Lys Leu Phe Lys Ser Gly Arg Leu Ser Pro Glu Trp Asp Val Met Gly
500 505 510
Cys Val Val Asn Ile Pro Ile Asp His Val Pro Leu Pro Ser Asp Val
515 520 525
Ser Phe Ala Val Ser Ser Glu Thr Leu Asn Met Lys Ala Pro Asn Gly
530 535 540
Thr Pro Ala Pro Val His Pro Asn Gln Gln Pro Ser Asp Glu Asn Thr
545 550 555 560
Leu Leu His Pro Tyr Ser Asp Gln Ser Tyr Tyr Ser Thr Asn Ser Asn
565 570 575

<210> 3
<211> 1743
<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (1)..(1743)

<400> 3
atg cct ttc cat gta gaa gga cta gta gcg att atc ctg ttc tac ctt 48
Met Pro Phe His Val Glu Gly Leu Val Ala Ile Ile Leu Phe Tyr Leu
1 5 10 15

ctt ata ttt ctg gtt gga ata tgg gct gca tgg aaa acc aaa aac agc 96
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
20 25 30

ggt aat gca gaa gaa cgc agc gaa gcc atc ata gtt ggg ggc cga gac 144
Gly Asn Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp

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        35                  40                  45

att ggt ttg ttg gtt ggt ggt ttt acc atg aca gcc acc tgg gtt gga   192
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
     50                  55                  60

gga ggt tac atc aac ggg aca gct gaa gca gtt tat ggg cca ggt tgt   240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
 65                  70                  75                  80

ggt cta gct tgg gct cag gca ccc att gga tat tct ctg agt ctg att   288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                 85                  90                  95

tta ggt ggc ctg ttt ttt gca aaa cct atg cgt tcc aag gga tat gtg   336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110

act atg tta gac ccg ttt caa cag atc tat gga aag cgc atg ggt ggg   384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125

ctg ctg ttc atc cct gca ctg atg gga gag atg ttc tgg gct gca gca   432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140

att ttc tct gca tta ggg gct acc atc agc gta atc att gat gtg gat   480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160

gtg aac ata tcg gtc att gtc tcc gca ctc att gcc att ctt tat acc   528
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
                165                 170                 175

ctc gtg gga ggg ctc tac tct gtg gca tat act gat gtt gta cag cta   576
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Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
180 185 190

ttc tgc att ttt ata gga ttg tgg atc agt gtc cca ttt gcc ctg tca 624
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
195 200 205

cat cct gca gtc acc gac att gga ttc act gct gtg cat gct aaa tac 672
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
210 215 220

cag agt ccc tgg ctg gga acc att gaa tca gtt gaa gtc tac acc tgg 720
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225 230 235 240

ctt gat aat ttt ctg ttg ttg atg ctg ggt gga ata cca tgg caa gcc 768
Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
245 250 255

tac ttc cag agg gtc ctc tct tca tcg tca gcg acc tat gct cag gtg 816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
260 265 270

ctg tcc ttc ctg gca gct ttt ggg tgc ctg gtg atg gct cta cca gcc 864
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
275 280 285

att tgc att ggg gcc att gga gcc tcc aca gac tgg aac caa act gca 912
Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300

tat ggg ttt cca gat ccc aag acc aag gag gaa gca gac atg att ctc 960
Tyr Gly Phe Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305 310 315 320

```
ccg att gtt cta cag tac ctc tgc cct gtg tac att tcc ttc ttt ggg   1008
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
                325                 330                 335

ctt ggt gct gtt tct gct gct gtc atg tcc tcg gct gac tca tcc atc   1056
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
            340                 345                 350

cta tca gca agt tcc atg ttt gct cgg aat atc tac cag ctt tcc ttc   1104
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
        355                 360                 365

aga caa aat gca tca gac aag gaa att gtg tgg gtc atg agg atc act   1152
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
    370                 375                 380

gtg ttt gtg ttt gga gca tct gca aca gcc atg gcc ttg ctc acg aag   1200
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385                 390                 395                 400

act gtg tat ggg ctc tgg tac ctg agc tct gac ctt gtc tac atc atc   1248
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
                405                 410                 415

atc ttc cca cag ctg ctc tgt gta ctc ttc atc aaa gga acc aac act   1296
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
            420                 425                 430

tat ggg gca gtt gct ggt tat att ttt gga ctt ttc ctg aga att acc   1344
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
        435                 440                 445

gga gga gag cca tat cta tac ttg cag ccc tta atc ttc tac cct ggt   1392
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
    450                 455                 460
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tat tac cct gac aag aat ggt ata tac aat cag agg ttc cca ttt aaa 1440
Tyr Tyr Pro Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465 470 475 480

act ctc tcc atg gtt acc tca ttc ttt acc aac att tgt gtt tcc tat 1488
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
485 490 495

cta gcc aag tat cta ttt gaa agt gga acc ttg cct cca aaa tta gat 1536
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510

ata ttt gat gct gtt gtc tca agg cac agt gaa gag aac atg gac aag 1584
Ile Phe Asp Ala Val Val Ser Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525

acc att cta gtc aga aat gaa aac atc aaa tta aat gaa ctt gca cct 1632
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
530 535 540

gta aag cct cga cag agc cta acc ctc agt tca act ttc acc aat aaa 1680
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545 550 555 560

gag gct ctc ctt gat gtt gat tcc agt cca gag gga tct ggg act gaa 1728
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
565 570 575

gat aac tta caa tga 1743
Asp Asn Leu Gln
580

<210> 4

<211> 580
<212> PRT
<213> Rattus norvegicus

<400> 4

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Met Pro Phe His Val Glu Gly Leu Val Ala Ile Ile Leu Phe Tyr Leu
 1               5                  10                  15
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
            20                  25                  30
Gly Asn Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
        35                  40                  45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
    50                  55                  60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
65                  70                  75                  80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                85                  90                  95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
                165                 170                 175
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225                 230                 235                 240
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Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
245 250 255
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
260 265 270
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
275 280 285
Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300
Tyr Gly Phe Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305 310 315 320
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
405 410 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
420 425 430
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
435 440 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460
Tyr Tyr Pro Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465 470 475 480
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
485 490 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510
Ile Phe Asp Ala Val Val Ser Arg His Ser Glu Glu Asn Met Asp Lys

```
        515                 520                 525
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
    530                 535                 540
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545                 550                 555                 560
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
                565                 570                 575
Asp Asn Leu Gln
            580
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<210> 5
<211> 1743
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1743)

<400> 5

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atg gct ttc cat gtg gaa gga ctg ata gct atc atc gtg ttc tac ctt   48
Met Ala Phe His Val Glu Gly Leu Ile Ala Ile Ile Val Phe Tyr Leu
  1               5                  10                  15

cta att ttg ctg gtt gga ata tgg gct gcc tgg aga acc aaa aac agt   96
Leu Ile Leu Leu Val Gly Ile Trp Ala Ala Trp Arg Thr Lys Asn Ser
            20                  25                  30

ggc agc gca gaa gag cgc agc gaa gcc atc ata gtt ggt ggc cga gat   144
Gly Ser Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
        35                  40                  45

att ggt tta ttg gtt ggt gga ttt acc atg aca gct acc tgg gtc gga   192
```

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Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
     50                  55                  60

gga ggg tat atc aat ggc aca gct gaa gca gtt tat gta cca ggt tat   240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Val Pro Gly Tyr
 65                  70                  75                  80

ggc cta gct tgg gct cag gca cca att gga tat tct ctt agt ctg att   288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                 85                  90                  95

tta ggt ggc ctg ttc ttt gca aaa cct atg cgt tca aag ggg tat gtg   336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110

acc atg tta gac ccg ttt cag caa atc tat gga aaa cgc atg ggc gga   384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125

ctc ctg ttt att cct gca ctg atg gga gaa atg ttc tgg gct gca gca   432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140

att ttc tct gct ttg gga gcc acc atc agc gtg atc atc gat gtg gat   480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160

atg cac att tct gtc atc atc tct gca ctc att gcc act ctg tac aca   528
Met His Ile Ser Val Ile Ile Ser Ala Leu Ile Ala Thr Leu Tyr Thr
                165                 170                 175

ctg gtg gga ggg ctc tat tct gtg gcc tac act gat gtc gtt cag ctc   576
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190
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```
ttt tgc att ttt gta ggg ctg tgg atc agc gtc ccc ttt gca ttg tca   624
Phe Cys Ile Phe Val Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205

cat cct gca gtc gca gac atc ggg ttc act gct gtg cat gcc aaa tac   672
His Pro Ala Val Ala Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220

caa aag ccg tgg ctg gga act gtt gac tca tct gaa gtc tac tct tgg   720
Gln Lys Pro Trp Leu Gly Thr Val Asp Ser Ser Glu Val Tyr Ser Trp
225                 230                 235                 240

ctt gat agt ttt ctg ttg ttg atg ctg ggt gga atc cca tgg caa gca   768
Leu Asp Ser Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
                245                 250                 255

tac ttt cag agg gtt ctc tct tct tcc tca gcc acc tat gct caa gtg   816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
            260                 265                 270

ctg tcc ttc ctg gca gct ttc ggg tgc ctg gtg atg gcc atc cca gcc   864
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Ile Pro Ala
        275                 280                 285

ata ctc att ggg gcc att gga gca tca aca gac tgg aac cag act gca   912
Ile Leu Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
    290                 295                 300

tat ggg ctt cca gat ccc aag act aca gaa gag gca gac atg att tta   960
Tyr Gly Leu Pro Asp Pro Lys Thr Thr Glu Glu Ala Asp Met Ile Leu
305                 310                 315                 320

cca att gtt ctg cag tat ctc tgc cct gtg tat att tct ttc ttt ggt  1008
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
                325                 330                 335
```

```
ctt ggt gca gtt tct gct gct gtt atg tca tca gca gat tct tcc atc   1056
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
            340                 345                 350

ttg tca gca agt tcc atg ttt gca cgg aac atc tac cag ctt tcc ttc   1104
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
        355                 360                 365

aga caa aat gct tcg gac aaa gaa atc gtt tgg gtt atg cga atc aca   1152
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
    370                 375                 380

gtg ttt gtg ttt gga gca tct gca aca gcc atg gcc ttg ctg acg aaa   1200
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385                 390                 395                 400

act gtg tat ggg ctc tgg tac ctc agt tct gac ctt gtt tac atc gtt   1248
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Val
                405                 410                 415

atc ttc ccc cag ctg ctt tgt gta ctc ttt gtt aag gga acc aac acc   1296
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Val Lys Gly Thr Asn Thr
            420                 425                 430

tat ggg gcc gtg gca ggt tat gtt tct ggc ctc ttc ctg aga ata act   1344
Tyr Gly Ala Val Ala Gly Tyr Val Ser Gly Leu Phe Leu Arg Ile Thr
        435                 440                 445

gga ggg gag cca tat ctg tat ctt cag ccc ttg atc ttc tac cct ggc   1392
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
    450                 455                 460

tat tac cct gat gat aat ggt ata tat aat cag aaa ttt cca ttt aaa   1440
Tyr Tyr Pro Asp Asp Asn Gly Ile Tyr Asn Gln Lys Phe Pro Phe Lys
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465                 470                 475                 480

aca ctt gcc atg gtt aca tca ttc tta acc aac att tgc atc tcc tat   1488
Thr Leu Ala Met Val Thr Ser Phe Leu Thr Asn Ile Cys Ile Ser Tyr
                485                 490                 495

cta gcc aag tat cta ttt gaa agt gga acc ttg cca cct aaa tta gat   1536
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
            500                 505                 510

gta ttt gat gct gtt gtt gca aga cac agt gaa gaa aac atg gat aag   1584
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
        515                 520                 525

aca att ctt gtc aaa aat gaa aat att aaa tta gat gaa ctt gca ctt   1632
Thr Ile Leu Val Lys Asn Glu Asn Ile Lys Leu Asp Glu Leu Ala Leu
    530                 535                 540

gtg aag cca cga cag agc atg acc ctc agc tca act ttc acc aat aaa   1680
Val Lys Pro Arg Gln Ser Met Thr Leu Ser Ser Thr Phe Thr Asn Lys
545                 550                 555                 560

gag gcc ttc ctt gat gtt gat tcc agt cca gaa ggg tct ggg act gaa   1728
Glu Ala Phe Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
                565                 570                 575

gat aat tta cag tga                                               1743
Asp Asn Leu Gln
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Leu Ile Leu Leu Val Gly Ile Trp Ala Ala Trp Arg Thr Lys Asn Ser
            20                  25                  30
Gly Ser Ala Glu Glu Arg Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
        35                  40                  45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
    50                  55                  60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Val Pro Gly Tyr
65                  70                  75                  80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                85                  90                  95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160
Met His Ile Ser Val Ile Ile Ser Ala Leu Ile Ala Thr Leu Tyr Thr
                165                 170                 175
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190
Phe Cys Ile Phe Val Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205
His Pro Ala Val Ala Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220
Gln Lys Pro Trp Leu Gly Thr Val Asp Ser Ser Glu Val Tyr Ser Trp
225                 230                 235                 240
Leu Asp Ser Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
                245                 250                 255
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
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260 265 270
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Ile Pro Ala
275 280 285
Ile Leu Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300
Tyr Gly Leu Pro Asp Pro Lys Thr Thr Glu Glu Ala Asp Met Ile Leu
305 310 315 320
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380
Val Phe Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Val
405 410 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Val Lys Gly Thr Asn Thr
420 425 430
Tyr Gly Ala Val Ala Gly Tyr Val Ser Gly Leu Phe Leu Arg Ile Thr
435 440 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460
Tyr Tyr Pro Asp Asp Asn Gly Ile Tyr Asn Gln Lys Phe Pro Phe Lys
465 470 475 480
Thr Leu Ala Met Val Thr Ser Phe Leu Thr Asn Ile Cys Ile Ser Tyr
485 490 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525
Thr Ile Leu Val Lys Asn Glu Asn Ile Lys Leu Asp Glu Leu Ala Leu
530 535 540

Val Lys Pro Arg Gln Ser Met Thr Leu Ser Ser Thr Phe Thr Asn Lys
545 550 555 560
Glu Ala Phe Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
565 570 575
Asp Asn Leu Gln
580

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<212> DNA
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<220>
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ctt ata ttt ctg gtt gga ata tgg gct gca tgg aaa acc aaa aac agc 96
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
20 25 30

ggc aac cca gaa gag cac agt gaa gcc atc ata gtc ggg ggc cgt gac 144
Gly Asn Pro Glu Glu His Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
35 40 45

att ggt ttg ttg gtt ggt ggt ttt acc atg aca gcc acc tgg gtt gga 192
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
50 55 60

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gga ggc tac atc aat ggg aca gca gaa gca gtg tat ggg cca ggt tgt  240
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
 65                  70                  75                  80

ggt cta gct tgg gct cag gca ccc att gga tat tct ctg agt cta att  288
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                 85                  90                  95

tta ggt ggt ctg ttt ttt gcg aaa cct atg cgt tcc aag gga tat gtg  336
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110

act atg tta gac cca ttt caa cag atc tat gga aag cgc atg ggt ggg  384
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125

ctg ctc ttc atc cct gca ctg atg gga gag atg ttc tgg gct gca gca  432
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140

att ttc tct gca tta ggg gcc acc atc agc gtg atc att gat gtg gat  480
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160

gtg aac ata tcg gtc att gtc tct gca ctc att gcc att ctt tat acc  528
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
                165                 170                 175

cta gtg ggt ggg ctc tac tct gtg gca tat act gat gtt gtc cag cta  576
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190

ttc tgc att ttt ata gga ctg tgg atc agt gtc cct ttt gcc ctg tca  624
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205
```

```
cat cct gca gtc acc gac atc gga ttc aca gct gtg cat gct aaa tac   672
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220

cag agt ccc tgg ctg gga acc att gaa tca gtt gaa gtc tac acc tgg   720
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225                 230                 235                 240

ctt gat aat ttt ctg tta ttg atg ctg ggt gga atc cca tgg caa gcc   768
Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
                245                 250                 255

tac ttc cag agg gtc ctc tct tca tcc tca gcc acc tat gct cag gta   816
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
            260                 265                 270

ctg tcc ttc ctg gca gct ttt ggg tgc ctg gtg atg gct cta ccc gcc   864
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
        275                 280                 285

ata tgc ata gga gct att gga gct tcc aca gac tgg aac cag act gcc   912
Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
    290                 295                 300

tac ggg tat cca gat ccc aag act aag gag gaa gca gac atg att ctc   960
Tyr Gly Tyr Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305                 310                 315                 320

ccg atc gtt ctg cag tac ctc tgc cct gtg tac atc tcc ttc ttt ggg  1008
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
                325                 330                 335

ctt ggt gct gtt tca gct gct gtc atg tcc tca gct gac tcg tcc atc  1056
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
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                340                 345                 350

ctg tcg gcg agt tct atg ttt gct cgg aat atc tac cag ctt tcc ttc   1104
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
        355                 360                 365

aga caa aat gca tca gac aag gaa att gtg tgg gtc atg agg atc act   1152
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
    370                 375                 380

gtg ctt gtg ttc gga gca tct gca aca gcc atg gct ttg ctg acg aag   1200
Val Leu Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385                 390                 395                 400

act gtg tat ggg ctc tgg tac ctg agc tct gac ctt gtc tac atc atc   1248
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
                405                 410                 415

atc ttc cca cag ctg ctc tgt gta ctc ttc atc aaa gga acc aac act   1296
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
            420                 425                 430

tat ggg gca gtt gct ggt tat att ttt gga cta ttc ctg aga att act   1344
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
        435                 440                 445

gga gga gag cca tat cta tac ttg cag ccc tta atc ttc tac cct ggt   1392
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
    450                 455                 460

tat tac tct gac aag aat ggt ata tac aat cag agg ttc cca ttt aaa   1440
Tyr Tyr Ser Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465                 470                 475                 480

act ctc tcc atg gtt acc tca ttc ttt acc aac att tgt gtt tct tat   1488
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Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
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cta gcc aag tat cta ttt gaa agt gga acc ttg cct cca aaa tta gat   1536
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
            500                 505                 510

gta ttt gat gct gtt gtc gca agg cac agt gaa gag aac atg gac aag   1584
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
        515                 520                 525

acc att cta gtc aga aat gaa aat atc aaa tta aat gaa ctt gca cct   1632
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
    530                 535                 540

gtg aaa cct cgg cag agc cta acc ctc agt tca act ttc acc aat aag   1680
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
545                 550                 555                 560

gag gcc ctc ctt gat gtt gat tcc agt ccg gag ggg tct ggg act gaa   1728
Glu Ala Leu Leu Asp Val Asp Ser Ser Pro Glu Gly Ser Gly Thr Glu
                565                 570                 575

gat aac tta caa tga                                              1743
Asp Asn Leu Gln
            580
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<210> 8
<211> 580
<212> PRT
<213> Mus musculus

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Met Ser Phe His Val Glu Gly Leu Val Ala Ile Ile Leu Phe Tyr Leu

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 1               5                  10                  15
Leu Ile Phe Leu Val Gly Ile Trp Ala Ala Trp Lys Thr Lys Asn Ser
            20                  25                  30
Gly Asn Pro Glu Glu His Ser Glu Ala Ile Ile Val Gly Gly Arg Asp
        35                  40                  45
Ile Gly Leu Leu Val Gly Gly Phe Thr Met Thr Ala Thr Trp Val Gly
    50                  55                  60
Gly Gly Tyr Ile Asn Gly Thr Ala Glu Ala Val Tyr Gly Pro Gly Cys
65                  70                  75                  80
Gly Leu Ala Trp Ala Gln Ala Pro Ile Gly Tyr Ser Leu Ser Leu Ile
                85                  90                  95
Leu Gly Gly Leu Phe Phe Ala Lys Pro Met Arg Ser Lys Gly Tyr Val
            100                 105                 110
Thr Met Leu Asp Pro Phe Gln Gln Ile Tyr Gly Lys Arg Met Gly Gly
        115                 120                 125
Leu Leu Phe Ile Pro Ala Leu Met Gly Glu Met Phe Trp Ala Ala Ala
    130                 135                 140
Ile Phe Ser Ala Leu Gly Ala Thr Ile Ser Val Ile Ile Asp Val Asp
145                 150                 155                 160
Val Asn Ile Ser Val Ile Val Ser Ala Leu Ile Ala Ile Leu Tyr Thr
                165                 170                 175
Leu Val Gly Gly Leu Tyr Ser Val Ala Tyr Thr Asp Val Val Gln Leu
            180                 185                 190
Phe Cys Ile Phe Ile Gly Leu Trp Ile Ser Val Pro Phe Ala Leu Ser
        195                 200                 205
His Pro Ala Val Thr Asp Ile Gly Phe Thr Ala Val His Ala Lys Tyr
    210                 215                 220
Gln Ser Pro Trp Leu Gly Thr Ile Glu Ser Val Glu Val Tyr Thr Trp
225                 230                 235                 240
Leu Asp Asn Phe Leu Leu Leu Met Leu Gly Gly Ile Pro Trp Gln Ala
                245                 250                 255
Tyr Phe Gln Arg Val Leu Ser Ser Ser Ser Ala Thr Tyr Ala Gln Val
            260                 265                 270
Leu Ser Phe Leu Ala Ala Phe Gly Cys Leu Val Met Ala Leu Pro Ala
        275                 280                 285
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Ile Cys Ile Gly Ala Ile Gly Ala Ser Thr Asp Trp Asn Gln Thr Ala
290 295 300
Tyr Gly Tyr Pro Asp Pro Lys Thr Lys Glu Glu Ala Asp Met Ile Leu
305 310 315 320
Pro Ile Val Leu Gln Tyr Leu Cys Pro Val Tyr Ile Ser Phe Phe Gly
325 330 335
Leu Gly Ala Val Ser Ala Ala Val Met Ser Ser Ala Asp Ser Ser Ile
340 345 350
Leu Ser Ala Ser Ser Met Phe Ala Arg Asn Ile Tyr Gln Leu Ser Phe
355 360 365
Arg Gln Asn Ala Ser Asp Lys Glu Ile Val Trp Val Met Arg Ile Thr
370 375 380
Val Leu Val Phe Gly Ala Ser Ala Thr Ala Met Ala Leu Leu Thr Lys
385 390 395 400
Thr Val Tyr Gly Leu Trp Tyr Leu Ser Ser Asp Leu Val Tyr Ile Ile
405 410 415
Ile Phe Pro Gln Leu Leu Cys Val Leu Phe Ile Lys Gly Thr Asn Thr
420 425 430
Tyr Gly Ala Val Ala Gly Tyr Ile Phe Gly Leu Phe Leu Arg Ile Thr
435 440 445
Gly Gly Glu Pro Tyr Leu Tyr Leu Gln Pro Leu Ile Phe Tyr Pro Gly
450 455 460
Tyr Tyr Ser Asp Lys Asn Gly Ile Tyr Asn Gln Arg Phe Pro Phe Lys
465 470 475 480
Thr Leu Ser Met Val Thr Ser Phe Phe Thr Asn Ile Cys Val Ser Tyr
485 490 495
Leu Ala Lys Tyr Leu Phe Glu Ser Gly Thr Leu Pro Pro Lys Leu Asp
500 505 510
Val Phe Asp Ala Val Val Ala Arg His Ser Glu Glu Asn Met Asp Lys
515 520 525
Thr Ile Leu Val Arg Asn Glu Asn Ile Lys Leu Asn Glu Leu Ala Pro
530 535 540
Val Lys Pro Arg Gln Ser Leu Thr Leu Ser Ser Thr Phe Thr Asn Lys
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565 570 575

Asp Asn Leu Gln

580

Fig. 9

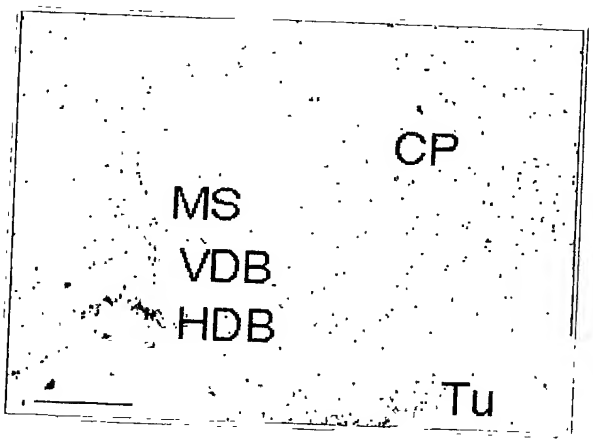

Fig. 10